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ORIGINAL FILED
DEC 12 2008
RICHARD W. WIEKING
CLERK, U.S. DISTRICT COURT,
NORTHERN DISTRICT OF CALIFORNIA

JCS

UNITED STATES DISTRICT COURT

FOR THE NORTHERN DISTRICT OF CALIFORNIA

THE CENTRAL INSTITUTE FOR EXPERIMENTAL ANIMALS, a Japanese corporation, Plaintiff, v. THE JACKSON LABORATORY, a Maine corporation Defendant.	Case No.: CV 08 5568 **COMPLAINT FOR PATENT INFRINGEMENT, TRADEMARK INFRINGEMENT, FALSE ADVERTISING, UNFAIR COMPETITION AND UNJUST ENRICHMENT** **DEMAND FOR JURY TRIAL**

Plaintiff, The Central Institute for Experimental Animals ("CIEA"), by its undersigned attorneys, brings this action against Defendant, The Jackson Laboratory ("Jackson Laboratory), for patent and trademark infringement, and unfair competition, and alleges as follows:

NATURE OF THE ACTION

1. This is a civil action alleging: (1) patent infringement in violation of the United States Patent Act, Title 35, United States Code, § 271(a); (2) trademark infringement in violation of the United States Trademark Act of 1946, as amended, 15 U.S.C. §§ 1114(1) and 1125(a); (3) unfair competition, false advertising and misrepresentation in violation of Section 43(a) of the United States Trademark Act of 1946, as amended, 15 U.S.C. § 1125(a); and related claims of unfair competition, false advertising, and unjust enrichment in violation of the laws of the state of California and the common law.

2. Plaintiff CIEA is a non-profit Japanese corporation with its principal place of business at 1430 Nogawa, Miyamae-ku, Kawasaki, Kanagawa, 216-0001 Japan. CIEA is engaged in the business of developing animal husbandry methods and animal models for use in biomedical and pharmaceutical research.

3. Upon information and belief, Defendant Jackson Laboratory is a privately-held, non-profit company incorporated and existing under the laws of the state of Maine, having its principal place of business at 600 Main Street, Bar Harbor, Maine 04609. Upon information and belief, Jackson Laboratory operates a production and distribution facility at 960 Riverside Parkway, West Sacramento, CA 95605, and markets, sells, licenses, and offers to sell and license within this judicial district products which infringe the patents and trademarks at issue.

JURISDICTION AND VENUE

4. This Court has jurisdiction over this action pursuant to 28 U.S.C. §§ 1331 and 1338 (a) and (b), and 15 U.S.C. § 1121.

5. This Court has supplemental jurisdiction over claims arising under California statutory and common law pursuant to 28 U.S.C. § 1367(a) because the state law claims are so related to the federal claims that they form part of the same case or controversy and derive from a common nucleus of operative facts.

6. Venue is proper in the court pursuant to 28 U.S.C. §§ 1391(b) and (c) because Jackson Laboratory markets, sells, licenses and offers to sell and license various products and

services within this judicial district, including its infringing product, and because a substantial part of the events giving rise to the claims occurred in this judicial district.

INTRADISTRICT ASSIGNMENT

7. Pursuant to Civil L.R. 3-2(c), this intellectual property action is excepted from assignment to a particular division.

FACTUAL ALLEGATIONS

CIEA and Its Patented Technology

8. CIEA was founded in 1952 by the renowned scientist Dr. Tatsuji Nomura. Dr. Nomura was a medical researcher performing research on infectious disease and was having trouble obtaining reproducible results due to the low quality of laboratory animals. As a result, he founded CIEA and dedicated his work to improving the level of medical and biomedical research by producing high quality research laboratory animals. Dr. Nomura has been a Person of Cultural Merit of Japan since 1997, and has received numerous awards, including Special Recognition Awards of the United States Food and Drug Administration in 1998 and in 2000.

9. Since that time, CIEA has become a world leader in the development of animal models and the establishment of concepts and methods for controlling the quality of laboratory animals, assuring reproducible animal experiments as "living assay systems."

10. CIEA has collaborated with research organizations all over the world and worked closely with such organizations as the World Health Organization, the United States National Institutes of Health, the United States Food and Drug Administration, and the International Council for Laboratory Animal Science.

11. For more than thirty years, one area of CIEA's expertise has been the development and production of various high-quality immunodeficient mouse strains that can be used for research of infectious disease, cancer and regenerative medicine. For example, in the early 1980's, CIEA scientists ushered in a new era of immunodeficient mouse models with the development of the "NOD/Shi mouse," a mouse strain lacking certain immune functions making it suitable for engraftment of human cells. Despite the advances made with this model, there were shortcomings

with the NOD/Shi mouse strain that rendered it less than optimal.

12. Collaborating with scientists from the internationally-recognized Tohoku University, including Dr. Kazuo Sugamura and CIEA scientists, including Drs. Mamoru Ito and Tatsuji Nomura, CIEA developed a new and severely immunodeficient mouse named the "NOG mouse."

13. The NOG mouse lacks T and B cells and natural-killer cells, and also has reduced macrophage and dendritic function, a long life span, and highly improved engraftment rates.

14. On December 5th, 2006 the United States Patent and Trademark Office ("USPTO") duly and lawfully issued U.S. patent No. 7,145,055 ("the '055 patent") entitled "Method of Producing a Mouse Model Suitable for the Engraftment, Differentiation, and Proliferation of Heterologous Cells, Mouse Produced by this Method, and Use of the Mouse." A copy of the '055 patent is attached as Exhibit A. The '055 patent issued to CIEA, as assignee of the inventors Mamoru Ito, et al.

15. The method disclosed in the '055 patent involves breeding two parental mouse strains. This method results in a derivative mouse strain, the NOG mouse, containing the genetic composition and physiologic characteristics claimed in the '055 patent.

16. CIEA's patented NOG mouse is unique because of its high tolerance to foreign cells and tissues in xeno-transplantation studies. The basis for this is the NOG mouse's severe immunodeficiency. Because of this important characteristic, human cells transplanted in the NOG mouse do not elicit a host immune response that would result in the destruction of those cells. Thus, the physiology and responsiveness to treatment of transplanted cells can be studied in vivo.

17. In general, the patented NOG mouse is used as a research tool to conduct studies on human physiology that are normally precluded because of ethical and/or safety concerns in human subjects. Specific applications of the mouse include (1) the basic study of the human immune system, (2) study of human disease mechanisms in vivo, and (3) pre-clinical trials of drugs. CIEA's patented NOG mouse is a research tool with broad applications and substantial utility in biomedical and pharmaceutical research and development.

18. After filing the application leading to the '055 patent, on December 14, 2001, CIEA

scientists submitted a manuscript entitled "NOD/SCID/Gamma-Null Mouse: An Excellent Recipient Mouse Model for Engraftment of Human Cells." This publication, which was published on July 5, 2002, describes in detail CIEA's patented NOG mouse and the process for producing it. A subsequent publication described the characterization of the mouse.

19. Both publications appear in peer-reviewed scientific journals with substantial readership in the scientific community. Indeed, CIEA's 2002 seminal report, which is mentioned above is cited over one-hundred thirty times in the scientific literature.

20. Upon information and belief, Jackson Laboratory's scientists had access to and reviewed the '055 patent and CIEA's publications regarding its NOG mouse.

21. Through an affiliate, CIEA markets and licenses its NOG mouse throughout the United States and in this judicial district. Since at least as early as June, 2004, CIEA, through one or more of its affiliates, has used in commerce the designation NOG as a trademark for its '055 patented mouse. In recognition of its exclusive trademark rights, the United States Patent and Trademark Office granted CIEA a federal trademark registration for the mark "NOG mouse," Reg. No. 3,118,040. (*See* Exhibit B). CIEA's trademark registration is valid and subsisting.

Jackson Laboratory's Infringing and Unlawful Activities

22. CIEA and Jackson Laboratory have known and respected each other for many years, and over those years, both companies have discussed various scientific and technological information of interest to the scientific community. CIEA shall continue to discuss scientific issues with Jackson Laboratory and other members of the scientific community since CIEA continues to be a leader in its field and is the innovator of the NOG mouse technology, which is an extremely important and valuable technology for CIEA.

23. In 2005—three years after CIEA's publication of its NOG mouse—Dr. Leonard Shultz, an investigator at Jackson Laboratory, published a paper disclosing Jackson Laboratory's production of an immunodeficient mouse. The mouse described in the publication is covered by the claims of the '055 patent (referred to as Jackson Laboratory's "Immunodeficient Mouse"). Dr. Schultz's paper is attached as Exhibit C. Since that time, Jackson Laboratory has used, without

permission, CIEA's NOG trademark in referring to its Immunodeficient Mouse, and has recently announced that it has adopted "NSG" as the name for its mouse.

24. More specifically, upon information and belief, Jackson Laboratory produced its Immunodeficient Mouse by genetically crossing a NOD-scid mouse with a mouse bearing a homozygous mutation in the IL2-R-gamma gene.

25. Upon information and belief, the mutation in the IL2-R-gamma gene results in the absence of expression of wild-type IL2-R-gamma protein.

26. Upon information and belief, the male progeny of this IL2-R-gamma mouse crossing are back-crossed to the parental NOD-scid mouse.

27. Upon information and belief, Jackson Laboratory's process of producing its Immunodeficient Mouse is covered by the claims in the '055 patent.

28. Upon information and belief, Jackson Laboratory's Immunodeficient Mouse has neither functional T-cells nor functional B-cells.

29. Upon information and belief, Jackson Laboratory's Immunodeficient Mouse has reduced macrophage function relative to the parental NOD-scid mouse.

30. Upon information and belief, Jackson Laboratory's Immunodeficient Mouse has reduced dendritic function relative to the parental NOD-scid mouse.

31. Upon information and belief, Jackson Laboratory's Immunodeficient Mouse, when transplanted with human hematopoietic stem cells, supports efficient differentiation and proliferation of said stem cells without eliminating them.

32. Upon information and belief, all the characteristics of Jackson Laboratory's Immunodeficient Mouse described above in paragraphs 28 - 31 are covered in claims of CIEA's '055 patent.

33. Soon after Dr. Schultz published his paper in 2005, Jackson Laboratory began marketing its Immunodeficient Mouse. Upon information and belief, Jackson Laboratory has marketed itself and represented to consumers, scientists and members of the trade that it is the originator and innovator of an immunodeficient mouse having all of the attributes claimed in CIEA's

'055 patent.

34. In his 2005 paper, Dr. Schultz characterizes Jackson Laboratory's Immunodeficient Mouse as "a new genetic stock of IL2-R common gamma-chain deficient NOD/LtSz-scid mice." Dr. Schultz also states that Jackson Laboratory's Immunodeficient Mouse engrafted with human stem cells "provide[s] a new in vivo long-lived model of robust multi-lineage human HSC engraftment."

35. Further, in reference to its Immunodeficient Mouse, Jackson Laboratory has stated that it created "A new mouse model that can support a human immune system." This accomplishment is credited to "The Jackson Laboratory's Dr. Leonard Shultz."

36. In referencing its "new" mouse, Jackson Laboratory has also stated in marketing materials that the Immunodeficient Mouse "has two major advantages over previous immunodeficient mouse models" and "superior ability to engraft human hematopoietic stem cells (HSCs) and differentiate them into the various cell subsets of the human immune system."

37. Upon information and belief, the statements referred to in paragraphs 34 - 36 above, were made with the knowledge of CIEA, its '055 patent and its NOG mouse.

38. Upon information and belief, Jackson Laboratory's statements are material, deceptive and false, and mislead or have a tendency to mislead consumers and members of the scientific community and public. Jackson Laboratory is not the originator or innovator behind the new immunodeficient mouse possessing the properties covered by the '055 patent.

39. Instead, it is CIEA and the named inventors on the '055 patent who are the innovators of the immunodeficient mouse claimed in the '055 patent.

40. Upon information and belief, Jackson Laboratory's false and/or misleading statements have harmed CIEA, its reputation and its ability to market its NOG mouse.

41. Moreover, upon information and belief, Jackson Laboratory's marketing materials have referred to its Immunodeficient Mouse by using CIEA's registered NOG trademark.

42. Upon information and belief, Jackson Laboratory's use of CIEA's NOG trademark in connection with the marketing, sale and commercialization of an immunodeficient mouse has caused and is likely to cause consumers and members of the public to believe that Jackson Laboratory's

Immunodeficient Mouse is CIEA's NOG mouse or that CIEA has licensed and approved of Jackson Laboratory's Immunodeficient Mouse, when CIEA has not done so.

43. Upon information and belief, Jackson Laboratory has traded, is trading, and will continue to trade on the goodwill of CIEA's NOG mark and patented technology. Jackson Laboratory's use of CIEA's NOG mark has reinforced the mistaken belief that Jackson Laboratory is the originator of the patented immunodeficient mouse claimed in the '055 patent.

44. In a publication entitled "Jax Notes" from the fall of 2008, Jackson Laboratory announced: "As of August 1, 2008, we have adopted the common name of 'NOD scid gamma', abbreviated 'NSG,' for this strain. We hope that this will alleviate confusion with another immunodeficient strain."

45. Upon information and belief, the statement "another immunodeficient strain" referred to in paragraph 44 above is a reference to CIEA's patented NOG mouse.

46. Jackson Laboratory's statement referred to in paragraph 44 above is an admission that it has used the NOG mark without permission from CIEA, and that its use of the NOG mark has caused confusion among consumers and is likely to continue doing so.

47. Upon information and belief, Jackson Laboratory's foregoing acts constitute deliberate and willful infringement of CIEA's '055 patent.

48. Upon information and belief, Jackson Laboratory's foregoing acts constitute deliberate and willful infringement of CIEA's NOG trademark.

49. Upon information and belief, Jackson Laboratory's foregoing marketing materials and statements about its Immunodeficient Mouse are deliberate and willful attempts to misrepresent to the public that Jackson Laboratory, not CIEA, is the originator and owner of the immunodeficient mouse claimed in the '055 patent.

50. As a proximate result of Jackson Laboratory's foregoing acts, CIEA has been damaged in an amount not yet determined, but which is in no event less than a reasonable royalty. Further, CIEA has been irreparably injured by these acts, and will continue to be damaged by such acts in the future unless Jackson Laboratory's acts are enjoined by this Court.

FIRST CLAIM FOR RELIEF

(PATENT INFRINGEMENT UNDER 35 U.S.C. § 271)

51. CIEA repeats and realleges every allegation in paragraphs 1-50 as if set forth fully here.

52. Jackson Laboratory has made, used, offered to sell and sold, and continues to make, use, offer to sell and sell its Immunodeficient Mouse throughout the United States and within this judicial district, and therefore it has infringed and continues to infringe the claims of the '055 patent under 35 U.S.C. § 271(a), either literally or under the doctrine of equivalents.

53. Jackson Laboratory has deliberately and knowingly marketed its Immunodeficient Mouse for proscribed uses that are claimed in the '055 patent. These acts constitute willful, contributory and induced infringement of the '055 patent pursuant to 35 U.S.C. §§ 271(b) and (c).

54. Jackson Laboratory's acts constitute willful infringement of the '055 patent pursuant to 35 U.S.C. § 271(a).

55. As a result of Jackson Laboratory's acts, CIEA has suffered damages in an amount to be determined, but in no event less than a reasonable royalty.

56. Jackson Laboratory's acts have caused irreparable harm and damage to CIEA for which CIEA has no adequate remedy at law.

SECOND CLAIM FOR RELIEF

(TRADEMARK INFRINGEMENT UNDER 15 U.S.C. § 1114(1))

57. CIEA repeats and realleges every allegation in paragraphs 1-56 as if set forth fully here.

58. Jackson Laboratory has used the trademark NOG in commerce in the marketing, sale and commercialization of its Immunodeficient Mouse in a manner that has caused and is likely to cause confusion among consumers and members of the public to believe that Jackson Laboratory's Immunodeficient Mouse is CIEA's NOG mouse or that CIEA has licensed and approved of Jackson

Laboratory's Immunodeficient Mouse, when CIEA has not done so.

59. Indeed, Jackson Laboratory has admitted that its use of the NOG trademark has caused confusion and is likely to cause confusion among consumers.

60. Jackson Laboratory's acts constitute willful infringement of CIEA's federally-registered trademark pursuant to 15 U.S.C. § 1114(1).

61. As a result of Jackson Laboratory's acts, CIEA has suffered damages in an amount to be determined at trial.

62. Jackson Laboratory's acts have caused irreparable harm and damage to CIEA for which CIEA has no adequate remedy at law.

THIRD CLAIM FOR RELIEF

(TRADEMARK INFRINGEMENT/FALSE DESIGNATION OF ORIGIN UNDER 15 U.S.C. § 1125(A))

63. CIEA repeats and realleges every allegation in paragraphs 1-62 as if set forth fully here.

64. Jackson Laboratory has used the NOG trademark in the marketing, sale and commercialization of its Immunodeficient Mouse in a manner that has caused and is likely to cause confusion among consumers and members of the public to believe that Jackson Laboratory's Immunodeficient Mouse is CIEA's NOG mouse or that CIEA has licensed and approved of Jackson Laboratory's mouse, when CIEA has not done so.

65. Indeed, Jackson Laboratory has admitted that its use of the NOG trademark has caused confusion and is likely to cause confusion among consumers.

66. Jackson Laboratory's acts are deliberate and intentional and constitute unfair competition and willful infringement of CIEA's trademark pursuant to 15 U.S.C. § 1125(a).

67. As a result of Jackson Laboratory's acts, CIEA has suffered damages in an amount to be determined at trial.

68. Jackson Laboratory's acts have caused irreparable harm and damage to CIEA for which CIEA has no adequate remedy at law.

FOURTH CLAIM FOR RELIEF

(FALSE ADVERTISING, MISREPRESENTATION

AND UNFAIR COMPETITION UNDER 15 U.S.C. § 1125(A))

69. CIEA repeats and realleges every allegation in paragraphs 1-68 as if set forth fully here.

70. Jackson Laboratory has made material, false statements and misrepresentations to consumers, scientists and members of the trade that it is the originator and innovator of a novel mouse that possesses properties covered under the claims of the '055 patent.

71. Jackson Laboratory has traded and is continuing to trade on the goodwill of CIEA and CIEA's patented NOG mouse.

72. Jackson Laboratory's deliberate and material, false and misleading statements have harmed CIEA, its reputation and its ability to market its patented NOG mouse.

73. Jackson Laboratory's acts constitute false advertising, misrepresentation, and unfair competition under 15 U.S.C. § 1125(a).

74. As a result of Jackson Laboratory's acts, CIEA has suffered damages in an amount to be determined at trial.

75. Jackson Laboratory's acts have caused irreparable harm and damage to CIEA for which CIEA has no adequate remedy at law.

FIFTH CLAIM FOR RELIEF

(UNFAIR COMPETITION — CAL. BUS. & PROF. CODE § 17200 ET SEQ.)

76. CIEA repeats and realleges every allegation in paragraphs 1-75 as if set forth fully here.

77. Jackson Laboratory's use of the NOG trademark and its material false and misleading statements and other wrongful acts described above have caused consumers, scientists, and members of the trade to believe mistakenly that Jackson Laboratory's Immunodeficient Mouse is associated with, sponsored by, or otherwise approved by CIEA and that it is Jackson Laboratory, not CIEA, who developed an immunodeficient mouse possessing the properties claimed in the '055 patent.

78. Jackson Laboratory's unlawful, unfair, and deceptive trade practices and other conduct described above, constitute unfair competition in violation of California Business and Professions Code § 17200 et seq.

79. As a result of Jackson Laboratory's acts, CIEA has suffered damages in an amount to be determined at trial.

80. Jackson Laboratory's acts have caused irreparable harm and damage to CIEA for which CIEA has no adequate remedy at law.

SIXTH CLAIM FOR RELIEF

(UNFAIR COMPETITION UNDER CALIFORNIA COMMON LAW)

81. CIEA repeats and realleges every allegation in paragraphs 1-80 as if set forth fully here.

82. Jackson Laboratory's use of the NOG trademark and its material false and misleading statements and other wrongful acts described above have caused consumers, scientists, and members of the trade to believe mistakenly that Jackson Laboratory's Immunodeficient Mouse is associated with, sponsored by, or otherwise approved by CIEA and that it is Jackson Laboratory, not CIEA, who developed an immunodeficient mouse possessing the properties claimed in the '055 patent.

83. Jackson Laboratory's acts are deliberate and willful, and it has traded and continues to trade in an unlawful manner on the goodwill of CIEA, its excellent reputation and its patented NOG mouse.

84. Jackson Laboratory's acts and conduct constitute unfair competition pursuant to the common law of California.

85. As a result of Jackson Laboratory's acts, CIEA has suffered damages in an amount to be determined at trial.

86. Jackson Laboratory's acts have caused irreparable harm and damage to CIEA for which CIEA has no adequate remedy at law.

SEVENTH CLAIM FOR RELIEF

(UNJUST ENRICHMENT UNDER CALIFORNIA LAW)

87. CIEA repeats and realleges every allegation in paragraphs 1-86 as if set forth fully here.

88. Jackson Laboratory's unauthorized use of the NOG trademark and its material false and misleading statements and other wrongful acts described above have caused consumers, scientists, and members of the trade to believe mistakenly that Jackson Laboratory's Immunodeficient Mouse is associated with, sponsored by, or otherwise approved by CIEA and that it is Jackson Laboratory, not CIEA, who developed an immunodeficient mouse possessing the properties claimed in the '055 patent.

89. Jackson Laboratory's acts are deliberate and willful, and it has knowingly traded and continues to trade in an unlawful manner on the goodwill of CIEA, its excellent reputation and its patented NOG mouse.

90. Jackson Laboratory has received and continues to receive benefits of its unauthorized use of CIEA's NOG trademark and CIEA's '055 patented invention, and from its trading on the goodwill and reputation of CIEA.

91. Jackson Laboratory has unjustly retained all of the benefits of its unauthorized use of CIEA's NOG trademark and CIEA's '055 patented invention.

92. As a result of Jackson Laboratory's acts and conduct, Jackson Laboratory has been unjustly enriched at CIEA's expense and to CIEA's detriment.

93. Jackson Laboratory's acts and conduct constitute unjust enrichment of Jackson Laboratory in violation of the common law of California.

94. CIEA is entitled to disgorgement of ill-gotten gains and to an award of damages in an amount to be proved at trial.

95. Jackson Laboratory's acts have caused irreparable harm and damage to CIEA for which CIEA has no adequate remedy at law.

PRAYER FOR RELIEF

WHEREFORE, CIEA respectfully requests this Court to enter a judgment against Jackson Laboratory as follows:

A. For judgment that Jackson Laboratory has infringed and continues to infringe the claims of the '055 patent under 35 U.S.C. §§ 271(a), (b) and (c);

B. For judgment that Jackson Laboratory's infringement of the '055 patent has been, and continues to be willful;

C. For judgment that Jackson Laboratory has infringed CIEA's NOG trademark in violation of 15 U.S.C. §§ 1114(1) and 1125(a);

D. For judgment that Jackson Laboratory has committed acts of false advertising, misrepresentation and unfair competition under 15 U.S.C. § 1125(a);

E. For judgment under California state statutory and common law for unfair competition and unjust enrichment;

F. For a preliminary and permanent injunction against Jackson Laboratory, its officers, directors, employees, agents, representatives, and all other persons in privity or acting in active concert with them,

(i) From making, using, selling, and licensing a mouse that infringes the claims of the '055 patent;

(ii) From using the NOG mark and any other colorable imitation in the marketing, sale and licensing of an immunodeficient mouse;

(iii) From making statements to consumers and members of the public that mislead or have a tendency to mislead consumers and members of the trade to believe that Jackson Laboratory is the innovator and originator of an immunodeficient mouse covered under the claims of the '055 patent.

G. For a mandatory injunction in the form of corrective advertising whereby Jackson Laboratory shall take all steps necessary to be approved by the Court, including the issuance of a press release and statements on Jackson Laboratory's Web site and marketing materials, alerting and communicating to consumers, scientists, and members of the trade that it is CIEA who is the innovator of mice covered under the claims of the '055 patent, not Jackson Laboratory;

H. For an award of damages adequate to compensate CIEA for the direct and/or indirect

patent infringement that has occurred in an amount to be proven at trial, but in no event less than a reasonable royalty;

I. For trebling of any and all damages awarded to CIEA by reason of Jackson Laboratory's willful infringement of the '055 patent, pursuant to 35 U.S.C. § 284;

J. For judgment that the case is exceptional under 35 U.S.C. § 285 and that the Court award CIEA its attorneys' fees;

K. For an award of damages pursuant to 15 U.S.C. § 1117(a) resulting from Jackson Laboratory's infringement of CIEA's NOG trademark, including, but not limited to, an award of Jackson Laboratory's profits, if any, and/or CIEA's damages;

L. For judgment that Jackson Laboratory's infringement of CIEA's NOG trademark was willful, and therefore, CIEA is entitled to reasonable attorneys' fees pursuant to 15 U.S.C. § 1117(a);

M. For a monetary award disgorging Jackson Laboratory's of ill-gotten gains resulting from its unjust enrichment at the expense of CIEA in an amount to be proven at trial;

N. For an award of CIEA's actual damages and punitive damages to be proven at trial;

O. For an assessment of prejudgment and post-judgment interest and costs against Jackson Laboratory for infringement; and

P. For such other and further relief both in law and equity to which CIEA is entitled.

Dated: December 12, 2008

KRIEG, KELLER, SLOAN, REILLEY & ROMAN LLP

By: Ken Kell

Kenneth E. Keller
Attorneys for Plaintiff Central Institute for Experimental Animals

DEMAND FOR JURY TRIAL

Pursuant to Rule 38(b) of the Federal Rules of Civil Procedure, demand is hereby made for trial by jury on all issues triable to a jury.

Dated: December 12, 2008

KRIEG, KELLER, SLOAN, REILLEY & ROMAN LLP

By: Ken Keller

Kenneth E. Keller
Attorneys for Plaintiff Central Institute for Experimental Animals

EXHIBIT A

US007145055B2

(12) **United States Patent**
Ito et al.

(10) **Patent No.:** **US 7,145,055 B2**
(45) **Date of Patent:** **Dec. 5, 2006**

(54) **METHOD OF PRODUCING A MOUSE SUITABLE FOR THE ENGRAFTMENT, DIFFERENTIATION AND PROLIFERATION OF HETEROLOGOUS CELLS, MOUSE PRODUCED BY THIS METHOD AND USE OF THE MOUSE**

(75) Inventors: **Mamoru Ito**, Kanagawa (JP); **Kimio Kobayashi**, Kanagawa (JP); **Tatsutoshi Nakahata**, Kyoto (JP); **Koichiro Tsuji**, Tokyo (JP); **Sonoko Habu**, Tokyo (JP); **Yoshio Koyanagi**, Chiba (JP); **Naoki Yamamoto**, Tokyo (JP); **Kazuo Sugamura**, Miyagi (JP); **Kiyoshi Ando**, Kanagawa (JP); **Tatsuji Nomura**, Tokyo (JP)

(73) Assignee: **Central Institute for Experimental Animals**, Kanagawa (JP)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 198 days.

(21) Appl. No.: **10/221,549**

(22) PCT Filed: **Oct. 25, 2001**

(86) PCT No.: **PCT/JP01/09401**

§ 371 (c)(1),
(2), (4) Date: **Sep. 10, 2002**

(87) PCT Pub. No.: **WO02/43477**

PCT Pub. Date: **Jun. 6, 2002**

(65) **Prior Publication Data**

US 2003/0182671 A1 Sep. 25, 2003

(30) **Foreign Application Priority Data**

Dec. 1, 2000 (JP) 2000-367296

(51) **Int. Cl.**
G01N 33/00 (2006.01)
C12N 15/01 (2006.01)
A01K 67/02 (2006.01)
A01K 67/027 (2006.01)

(52) **U.S. Cl.** **800/3**; 800/18; 800/21; 800/22

(58) **Field of Classification Search** 800/3, 800/18, 22

See application file for complete search history.

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(Continued)

Primary Examiner—Daniel M. Sullivan
(74) *Attorney, Agent, or Firm*—Ginger R. Dreger; Heller Ehrman LLP

(57) **ABSTRACT**

The present invention provides an immunodeficient mouse (NOG mouse) suitable for engraftment, differentiation and proliferation of heterologous cells, and a method of producing such a mouse. This mouse is obtained by backcrossing a C.B-17-scid mouse with an NOD/Shi mouse, and further backcrossing an interleukin 2-receptor γ-chain gene-knockout mouse with the thus backcrossed mouse. It is usable for producing a human antibody and establishing a stem cell assay system, a tumor model and a virus-infection model.

14 Claims, 28 Drawing Sheets

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Schultz, L. et al., "NOD LtSZ-Rad 1 null Mice: An Immunodeficient and Radioresistant Model for Engrafment of Human Hematolymphoid Cells, HIV Infection, and Adoptive Transfer of NOD Mouse Diabetogenic T Cells". *Journal of Immunology* 2496-2507 (2000).

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* cited by examiner

FIG.1

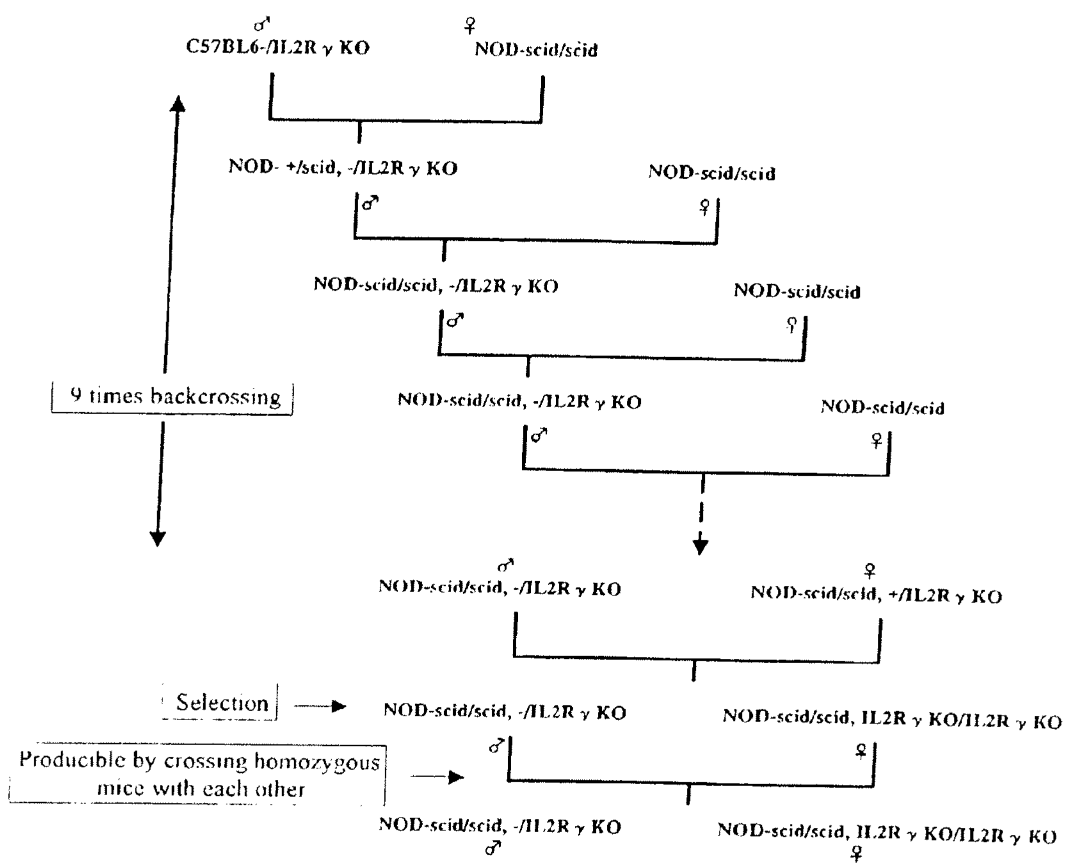

♂ C57BL6-/IL2R γ KO
♀ NOD-scid/scid
NOD- +/scid, -/IL2R γ KO
NOD-scid/scid
NOD-scid/scid, -/IL2R γ KO
NOD-scid/scid
9 times backcrossing
NOD-scid/scid, -/IL2R γ KO
NOD-scid/scid
♂ NOD-scid/scid, -/IL2R γ KO
♀ NOD-scid/scid, +/IL2R γ KO
Selection
NOD-scid/scid, -/IL2R γ KO
NOD-scid/scid, IL2R γ KO/IL2R γ KO
Producible by crossing homozygous mice with each other
NOD-scid/scid, -/IL2R γ KO ♂
NOD-scid/scid, IL2R γ KO/IL2R γ KO ♀

FIG.2A

Posttrans--plantation	mouse			hCD45+(%)	hCD45+(/μl)	hCD41+(%)	hCD41+(/μl)
4th week	I	8 mice	Mean	1.345	12.503	0.037	387.087
			SE	0.281	2.683	0.013	144.057
	II	8 mice	Mean	8.585	79.116	0.384	3096.365
			SE	1.668	21.701	0.138	647.664
8th week	I	8 mice	Mean	6.623	81.513	0.086	590.814
			SE	3.097	33.896	0.053	349.314
	II	8 mice	Mean	37.152	533.456	0.380	2422.976
			SE	7.324	99.283	0.112	547.911
12th week	I	7 mice	Mean	5.860	61.999	2.064	1510.084
			SE	2.395	22.302	1.308	769.132
	II	6 mice	Mean	24.086	530.097	2.586	2227.427
			SE	5.789	281.806	0.600	466.970

Mouse I: NOD/Shi-scid + antiAGM1
Mouse II: NOG

FIG.2B

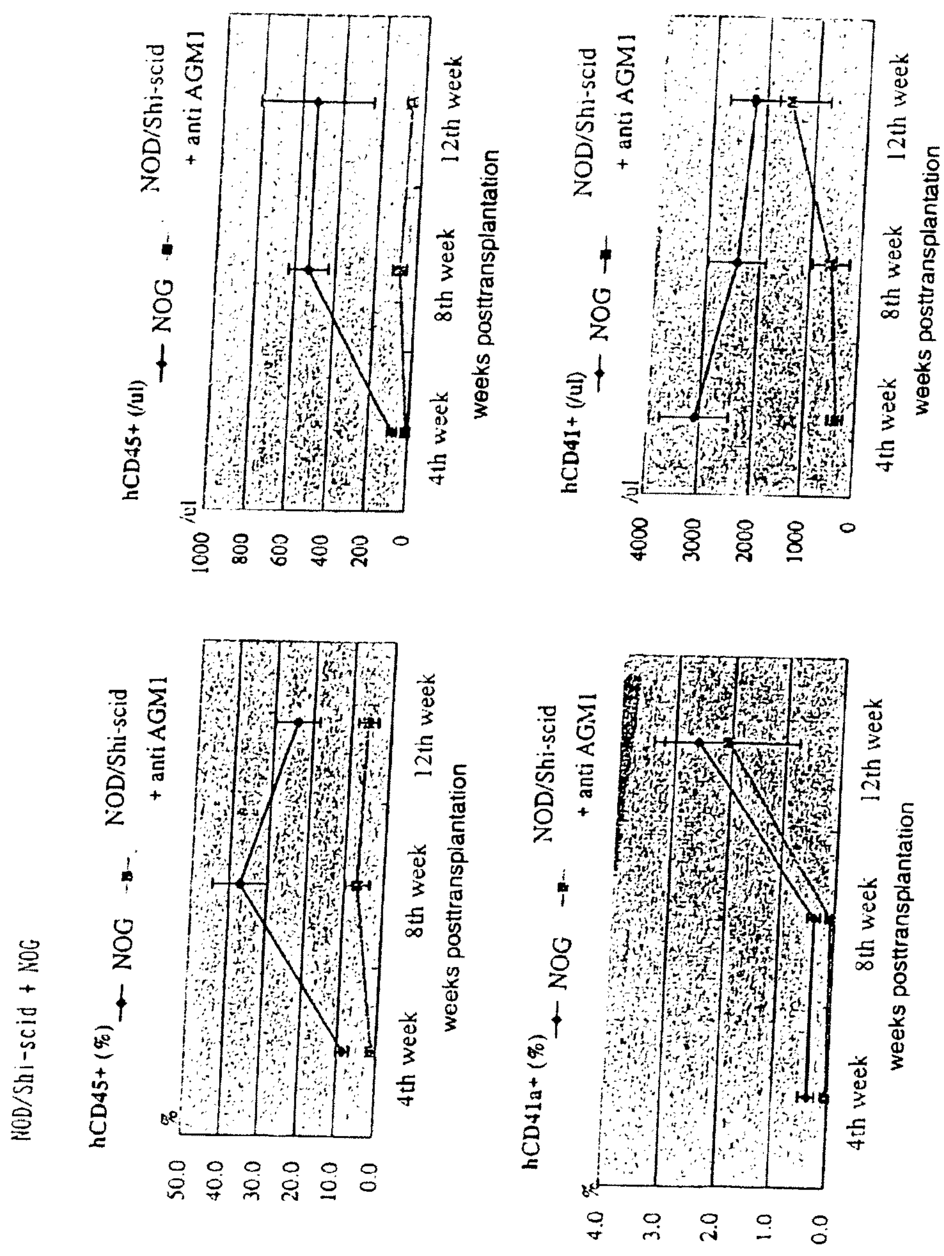

NOD/Shi-scid + NOG
hCD45+ (%)
NOG
NOD/Shi-scid + anti AGM1
50.0
40.0
30.0
20.0
10.0
0.0
%
4th week
8th week
12th week
weeks posttransplantation
hCD45+ (/ul)
NOG
NOD/Shi-scid + anti AGM1
1000
800
600
400
200
0
/ul
4th week
8th week
12th week
weeks posttransplantation
hCD41a+ (%)
NOG
NOD/Shi-scid + anti AGM1
4.0
3.0
2.0
1.0
0.0
%
4th week
8th week
12th week
weeks posttransplantation
hCD41+ (/ul)
NOG
NOD/Shi-scid + anti AGM1
4000
3000
2000
1000
0
/ul
4th week
8th week
12th week
weeks posttransplantation

FIG.3

		hCD45%	
		Bone marrow	Spleen
Mouse II	Mean SE	66.15 6.0	66.58 7.7
Mouse I	Mean SE	20.66 9.6	1.66 0.6

Mouse I : NOD/Shi-scid + antiAGM
Mouse II : NOG

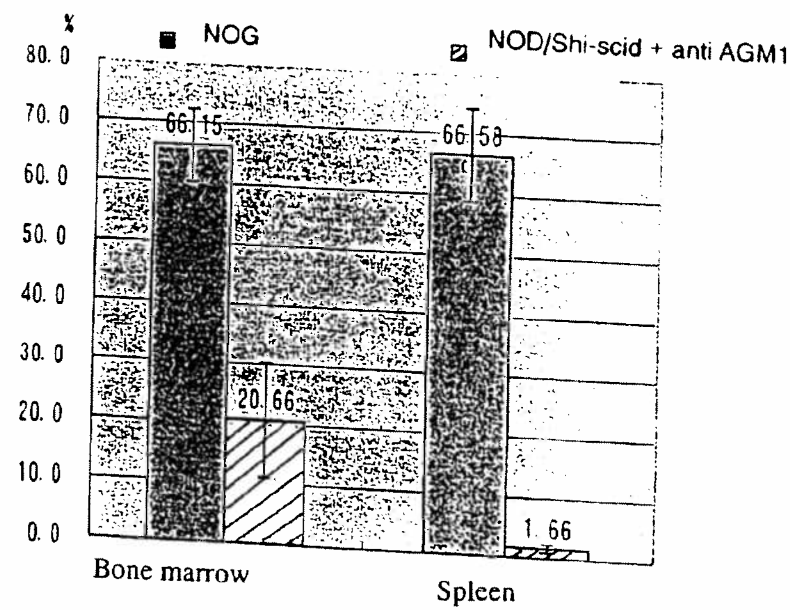


FIG.4

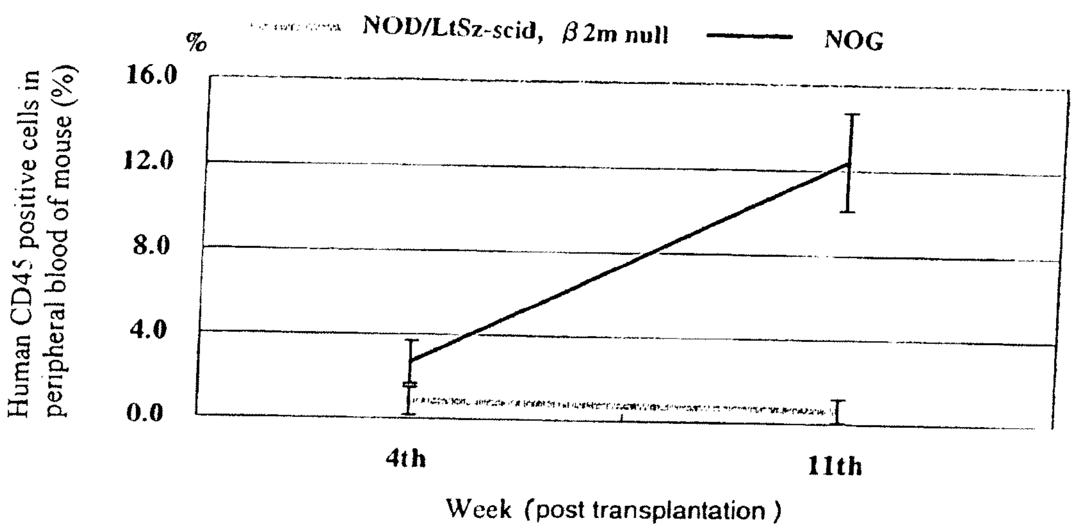

NOD/LtSz-scid, β2m null
NOG
%
16.0
12.0
8.0
4.0
0.0
Human CD45 positive cells in peripheral blood of mouse (%)
4th
11th
Week (post transplantation)

FIG.5A

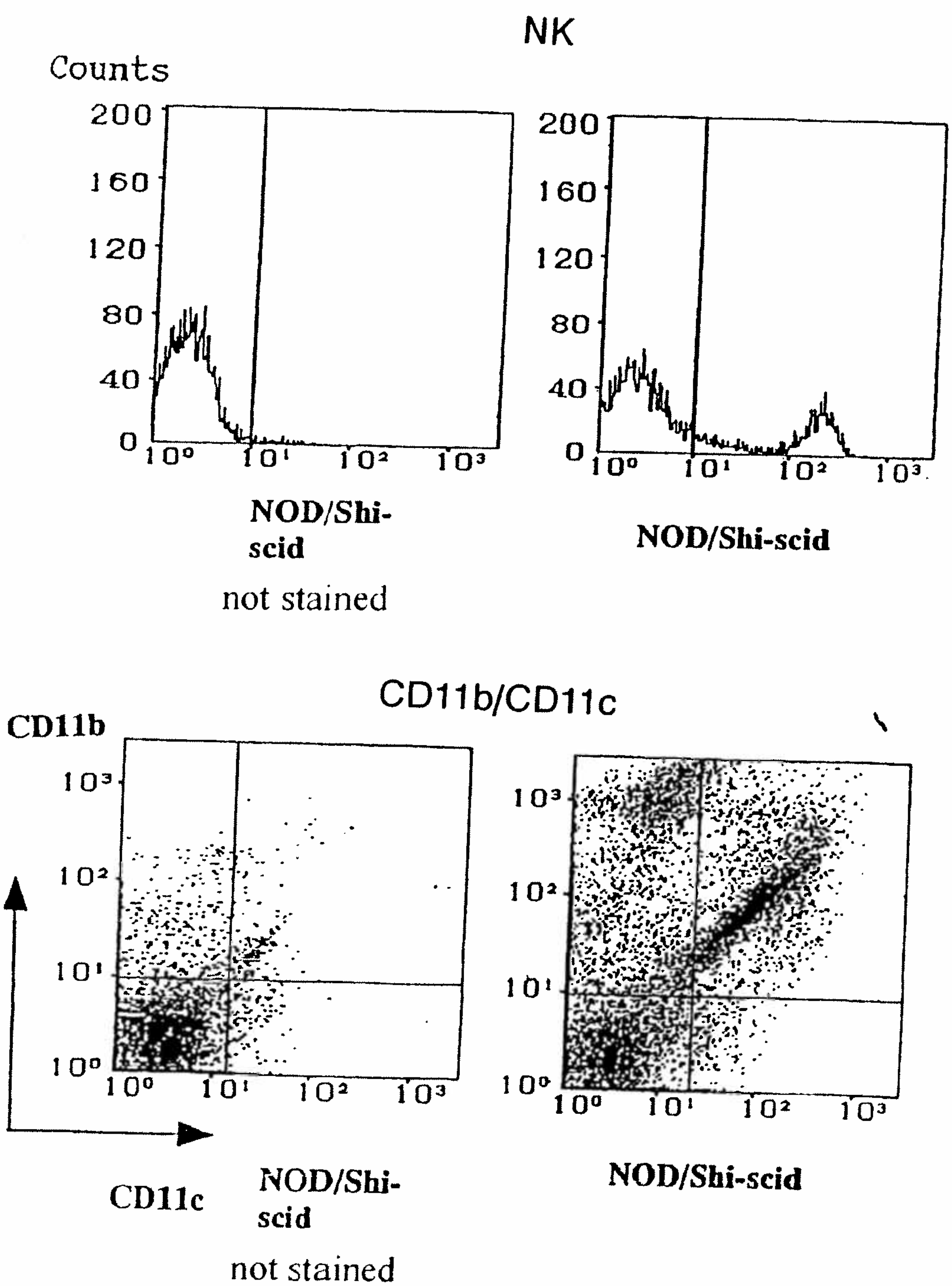

NK
Counts
200
160
120
80
40
0
10⁰ 10¹ 10² 10³
NOD/Shi-scid
not stained
NOD/Shi-scid
CD11b/CD11c
CD11b
10⁰ 10¹ 10² 10³
CD11c
NOD/Shi-scid
not stained
NOD/Shi-scid

FIG.5B

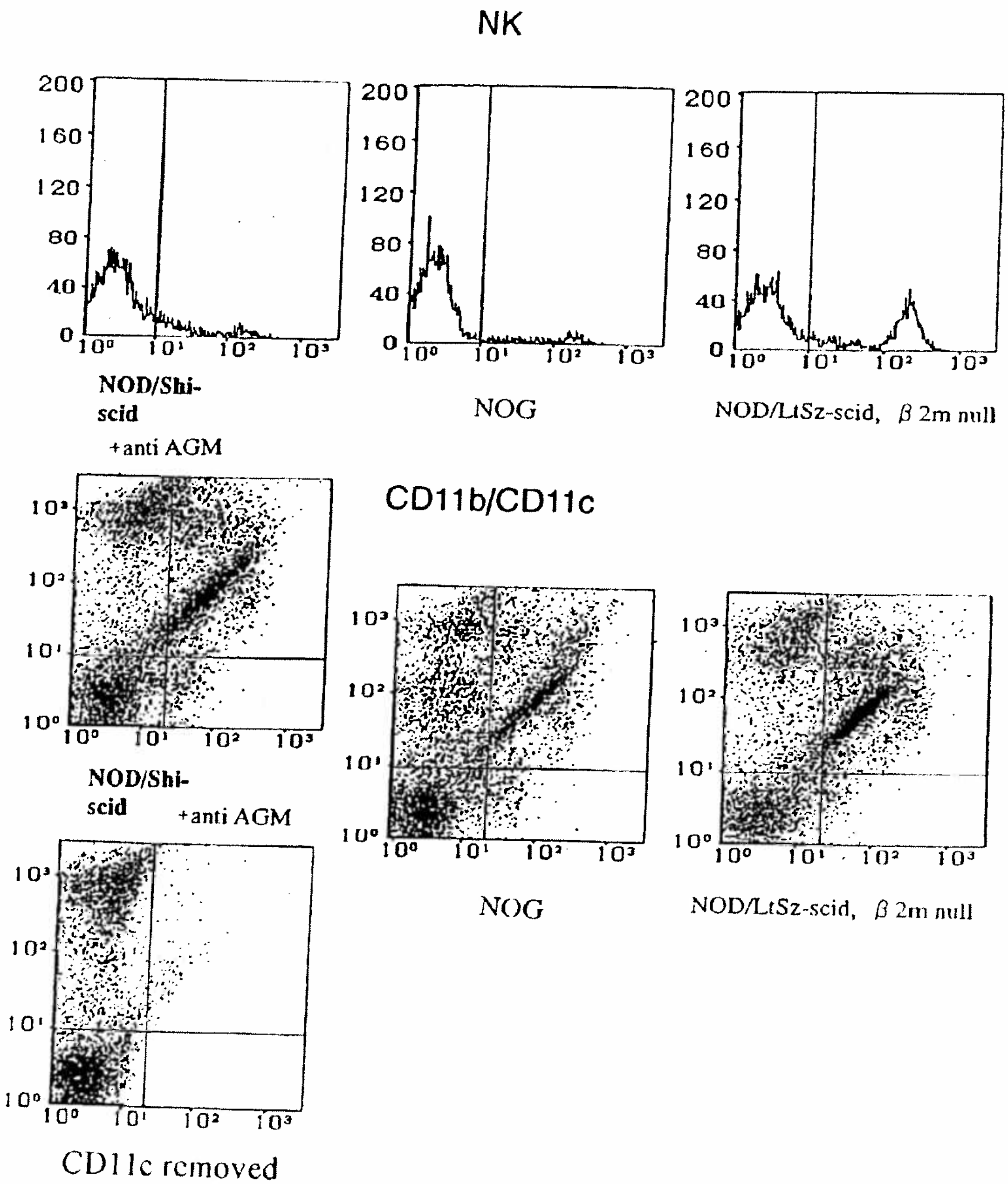

NK
200
160
120
80
40
0
10^0 10^1 10^2 10^3
NOD/Shi-scid
+anti AGM
NOG
NOD/LtSz-scid, β2m null
CD11b/CD11c
NOD/Shi-scid +anti AGM
NOG
NOD/LtSz-scid, β2m null
CD11c removed

FIG.6A

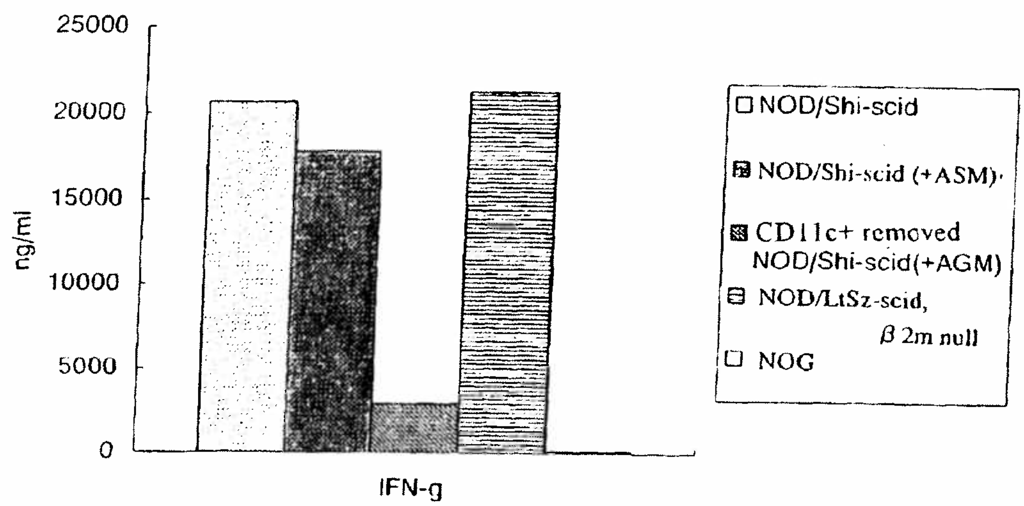

25000
20000
15000
10000
5000
0
ng/ml
IFN-g
NOD/Shi-scid
NOD/Shi-scid (+ASM)
CD11c+ removed NOD/Shi-scid(+AGM)
NOD/LtSz-scid, β 2m null
NOG

FIG.6B

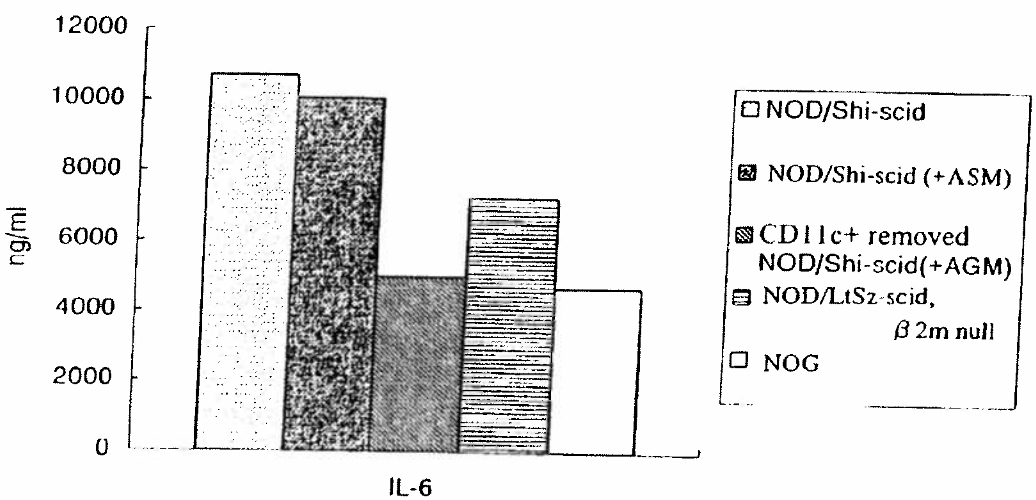

12000
10000
8000
6000
4000
2000
0
ng/ml
IL-6
NOD/Shi-scid
NOD/Shi-scid (+ASM)
CD11c+ removed NOD/Shi-scid(+AGM)
NOD/LtSz-scid, β2m null
NOG

FIG.6C

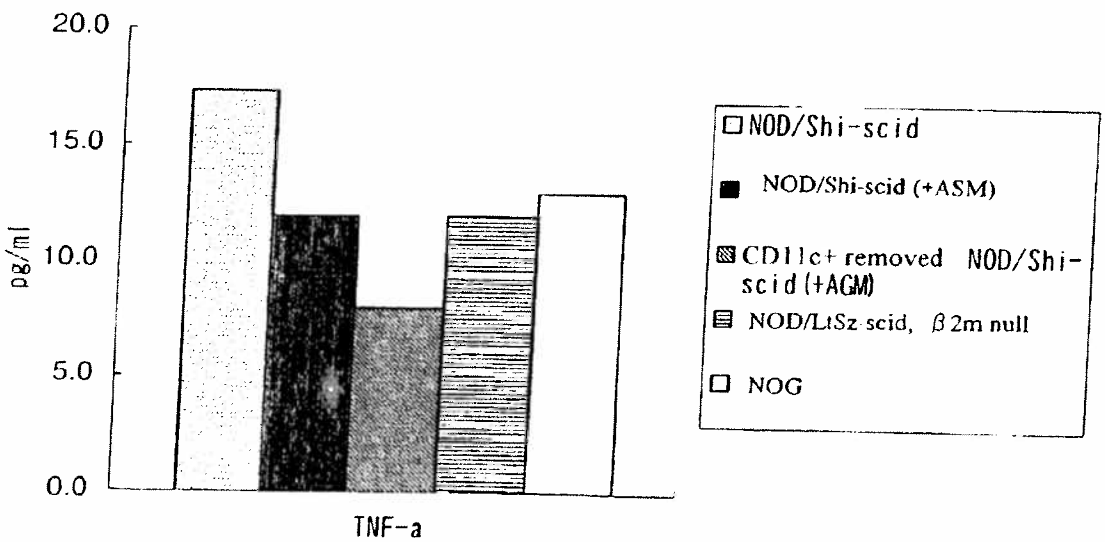
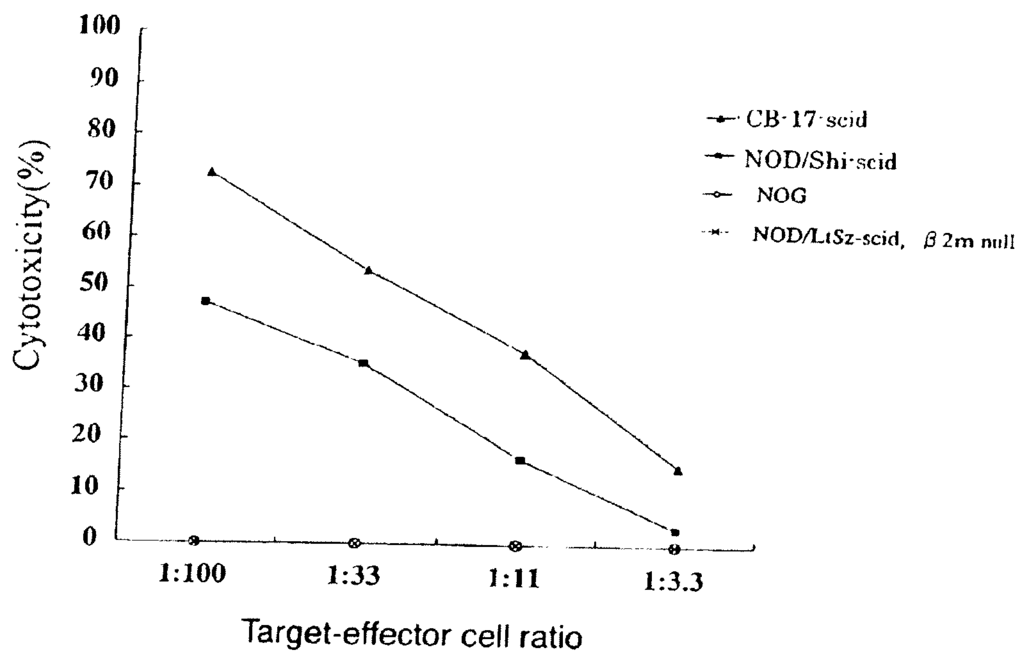

20.0
15.0
10.0
5.0
0.0
pg/ml
TNF-a
NOD/Shi-scid
NOD/Shi-scid (+ASM)
CD11c+ removed NOD/Shi-scid (+AGM)
NOD/LtSz-scid, β2m null
NOG

FIG.7



100
90
80
70
60
50
40
30
20
10
0
Cytotoxicity(%)
CB-17-scid
NOD/Shi-scid
NOG
NOD/LtSz-scid, β2m null
1:100
1:33
1:11
1:3.3
Target-effector cell ratio

FIG.8

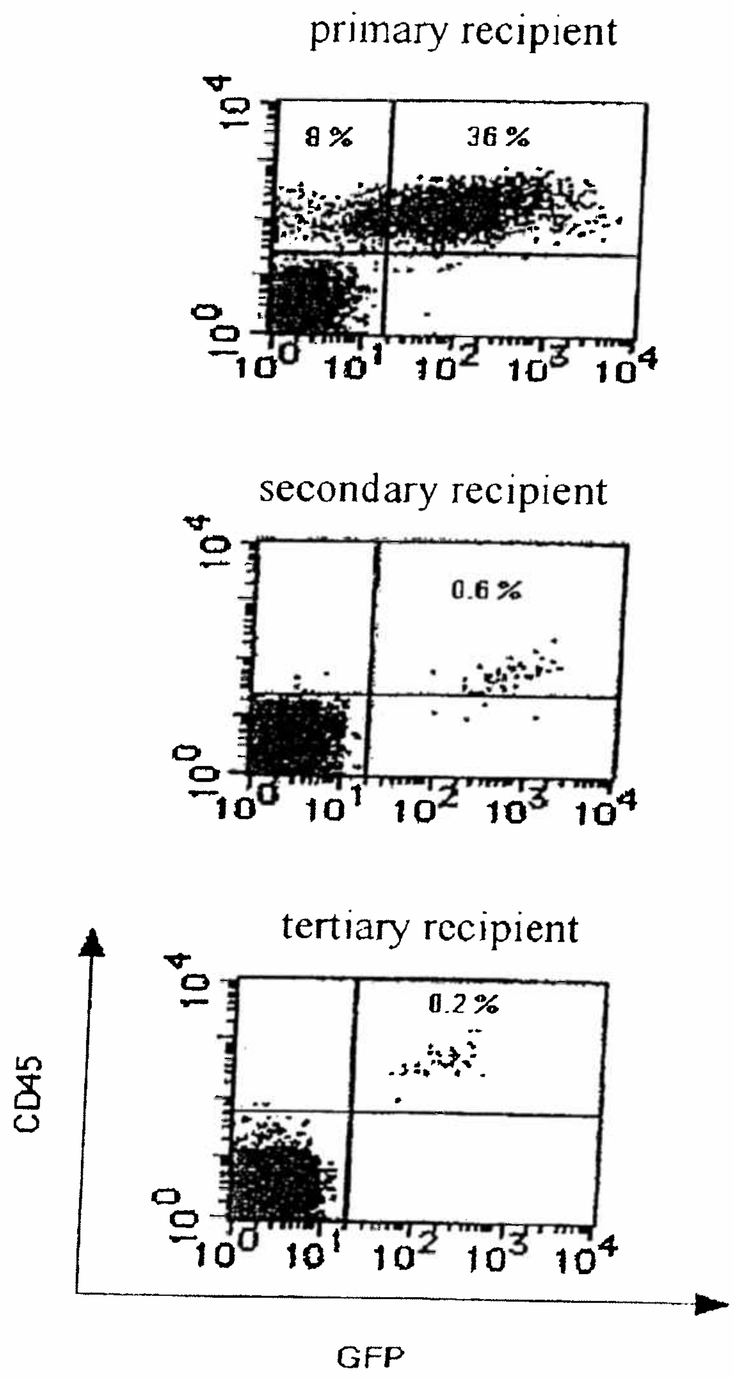
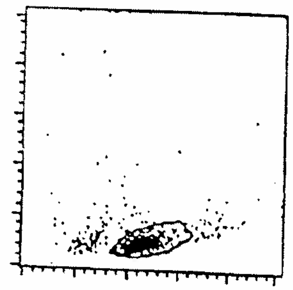

primary recipient
8 %
36 %
secondary recipient
0.6 %
tertiary recipient
0.2 %
CD45
GFP

FIG.9A Flowcytometric Analysis of thymic cells

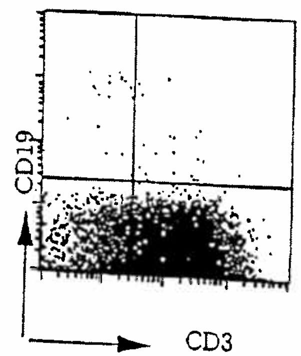
No of thymic cells = $2.1 \times 10^6 \times 2$ml



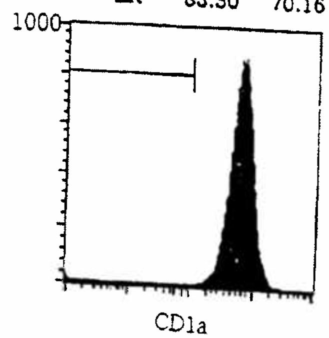
Gate	% Gated	% Total
G1	84.22	84.22
G2	94.33	94.33

G1 = lymphocyte
G2 = human $CD45^+$ cell

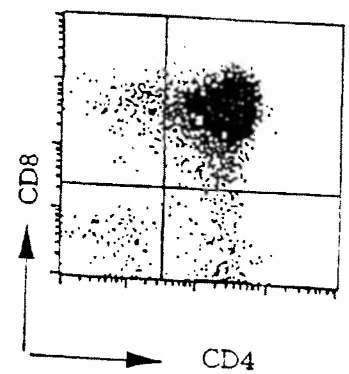
$CD34^+$: 5×10^5 cells transplanted



Quad	% Gated	% Total
UL	0.09	0.07
UR	0.12	0.10
LL	16.50	13.90
LR	83.30	70.16



Analysis on 11th week after transplanting



Quad	% Gated	% Total
UL	0.86	0.73
UR	97.26	82.48
LL	0.51	0.43
LR	1.37	1.17

Marker	% Gated	% Total
All	100.00	84.81
M1	1.14	0.97

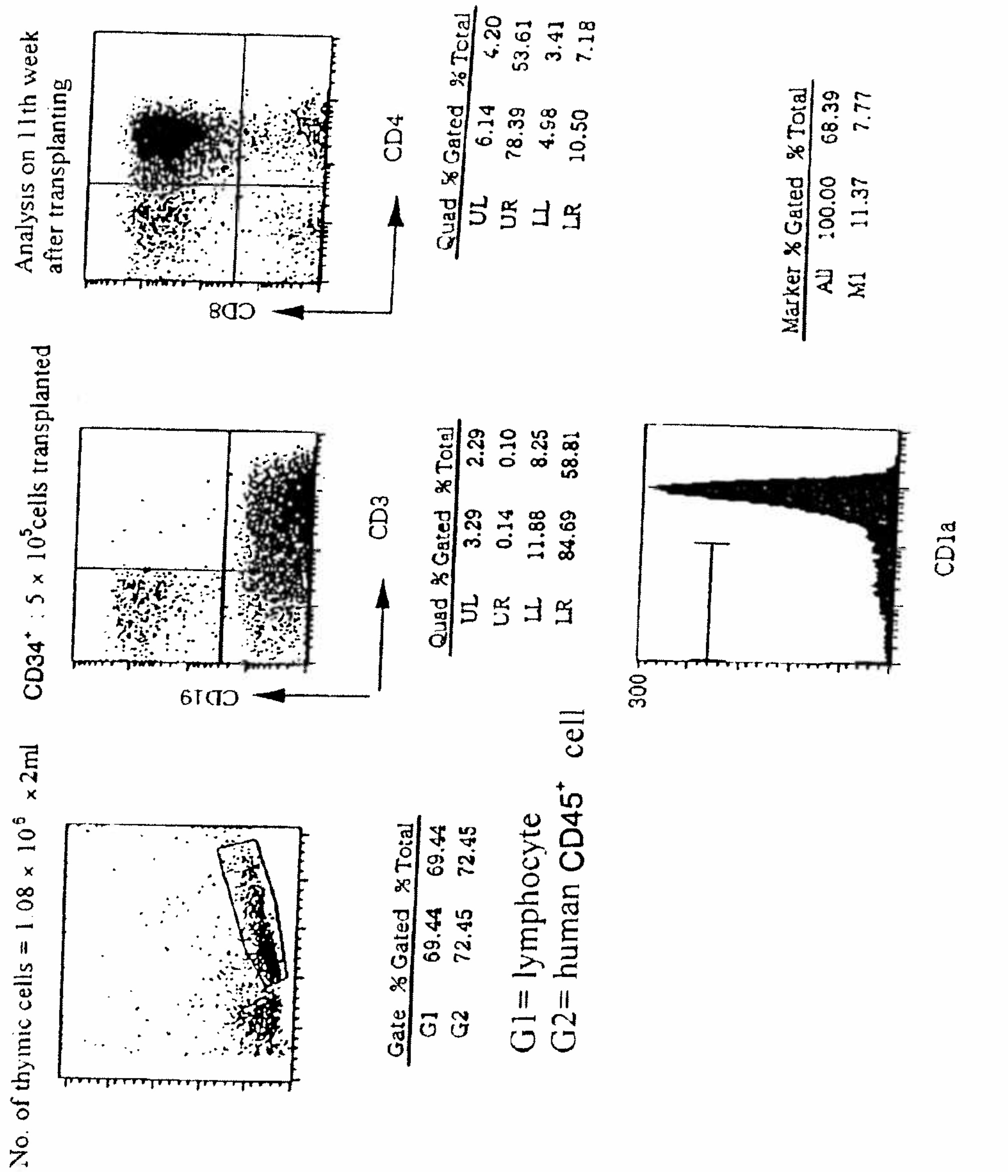

No. of thymic cells = 1.08 × 10^6 × 2ml
CD34+ : 5 × 10^5 cells transplanted
Analysis on 11th week after transplanting
Gate %Gated %Total
G1 69.44 69.44
G2 72.45 72.45
G1= lymphocyte
G2= human CD45+ cell
CD19
CD3
Quad %Gated %Total
UL 3.29 2.29
UR 0.14 0.10
LL 11.88 8.25
LR 84.69 58.81
CD8
CD4
Quad %Gated %Total
UL 6.14 4.20
UR 78.39 53.61
LL 4.98 3.41
LR 10.50 7.18
300
CD1a
Marker %Gated %Total
All 100.00 68.39
M1 11.37 7.77

FIG.9B

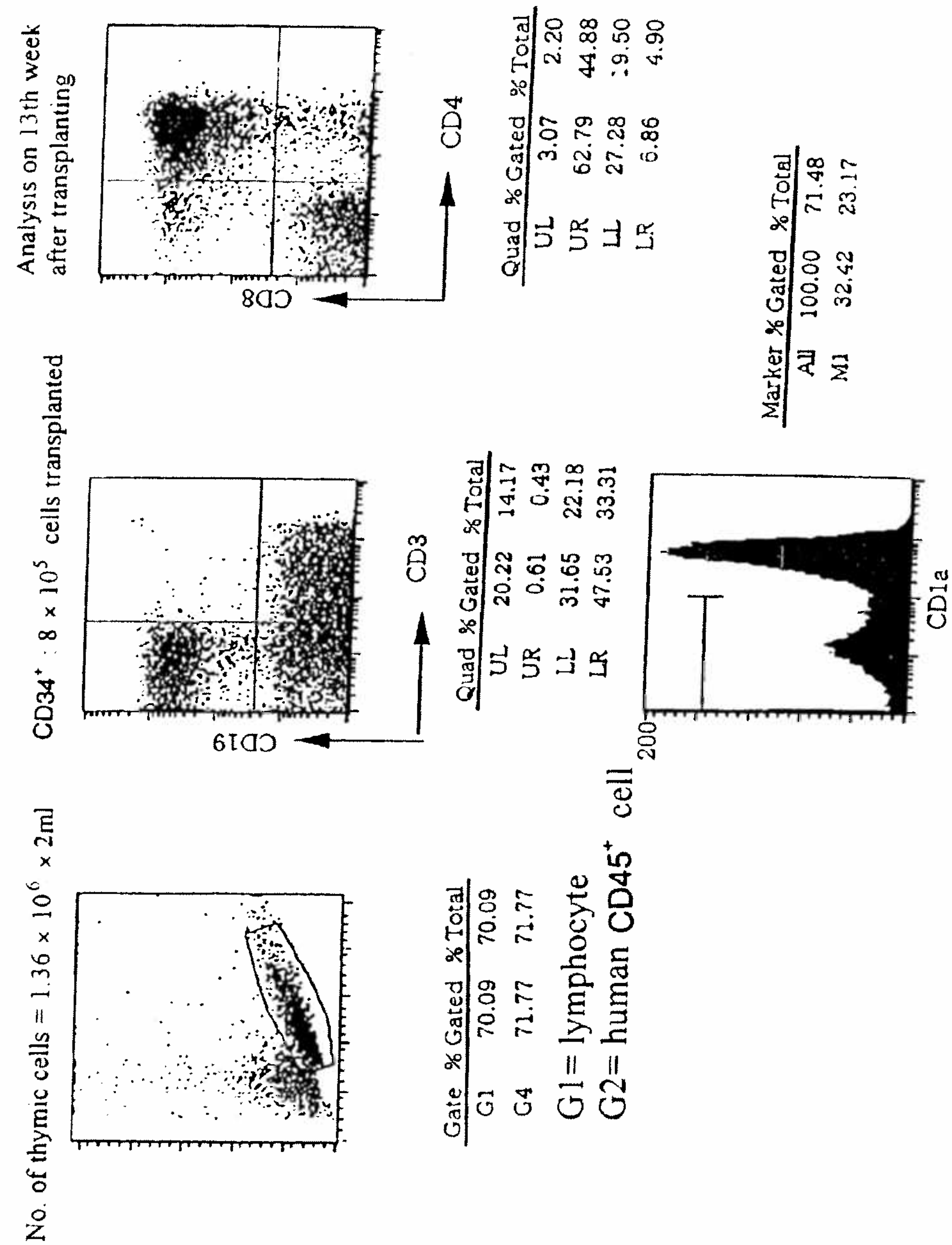
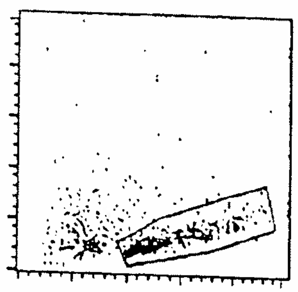

No. of thymic cells = 1.36 × 10^6 × 2ml
CD34+ : 8 × 10^5 cells transplanted
Analysis on 13th week after transplanting
Gate % Gated % Total
G1 70.09 70.09
G4 71.77 71.77
G1= lymphocyte
G2= human CD45+ cell
CD19
CD3
Quad % Gated % Total
UL 20.22 14.17
UR 0.61 0.43
LL 31.65 22.18
LR 47.53 33.31
200
CD1a
Marker % Gated % Total
All 100.00 71.48
M1 32.42 23.17
CD8
CD4
Quad % Gated % Total
UL 3.07 2.20
UR 62.79 44.88
LL 27.28 19.50
LR 6.86 4.90

FIG.10A

FIG.10B

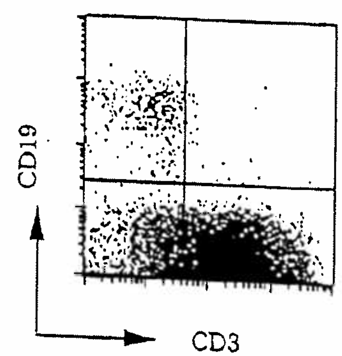
No. of thymic cells = 1.00 × 10^6 × 2ml



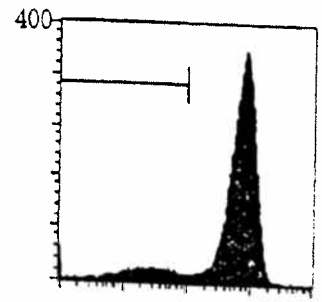
Gate	% Gated	% Total
G1	73.96	73.96
G2	77.26	77.26

G1 = lymphocyte
G2 = human $CD45^+$ cell

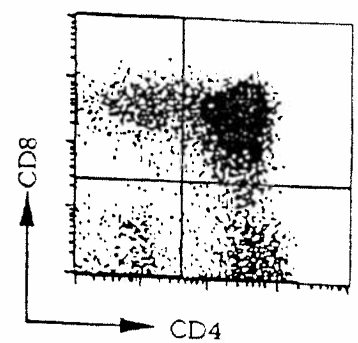
$CD34^+$: 5 × 10^5 cells transplanted



Quad	% Gated	% Total
UL	1.68	1.24
UR	0.13	0.10
LL	14.72	10.89
LR	83.47	61.73



Analysis on 13th week after transplanting



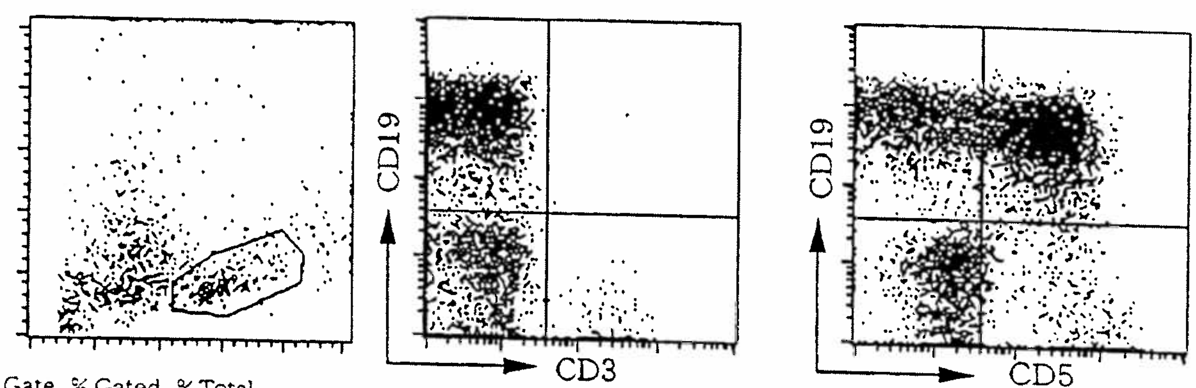
Quad	% Gated	% Total
UL	4.42	3.23
UR	80.85	59.02
LL	3.16	2.31
LR	11.56	8.44

Marker	% Gated	% Total
All	100.00	73.00
M1	10.19	7.43

FIG. 11A

Flowcytometric Analysis of spleen cells

No. of spleen cells = $5.02 \times 10^6 \times 5ml \times 2$



Gate	% Gated	% Total
G1	27.14	27.14
G2	31.01	31.01

G1 = lymphocyte
G2 = human $CD45^+$ cell

Quad	% Gated	% Total
UL	74.02	20.09
UR	0.08	0.02
LL	23.75	6.45
LR	2.15	0.58

Quad	% Gated	% Total
UL	27.82	7.84
UR	45.11	12.71
LL	19.53	5.50
LR	7.54	2.12

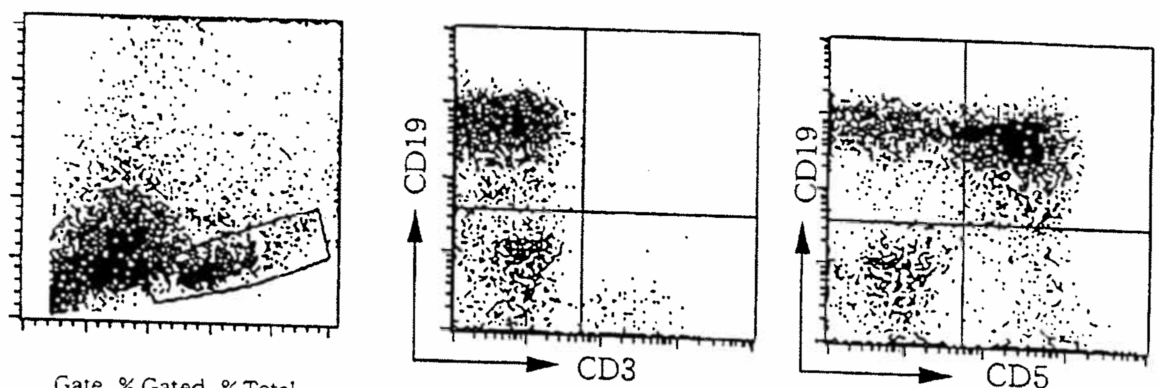
$CD34^+$: 5×10^5 cells transplanted

Analysis on 11th week after transplanting

FIG. 11B

Flowcytometric Analysis of thymic cells

No. of spleen cells = 2.48 $\times 10^6$ $\times$ 5ml $\times$ 2



Gate	% Gated	% Total
G1	19.59	19.59
G2	26.49	26.49

G1= lymphocyte

G2= human CD45$^+$ cell

Quad	% Gated	% Total
UL	73.24	14.35
UR	0.05	0.01
LL	22.66	4.44
LR	4.05	0.79

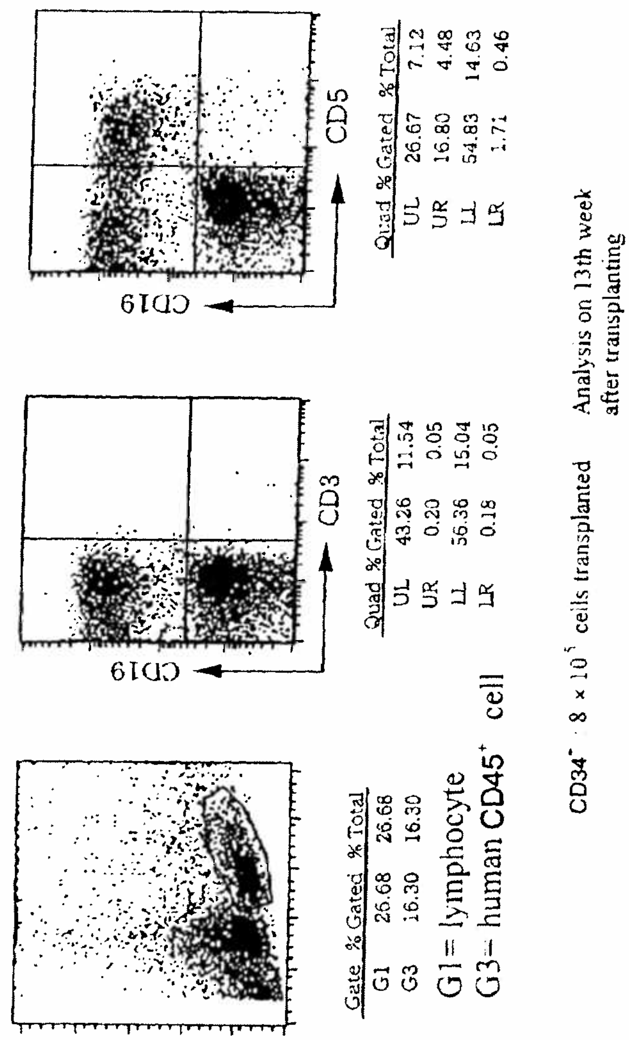
Quad	% Gated	% Total
UL	26.67	4.95
UR	47.71	8.85
LL	16.36	3.03
LR	9.26	1.72

CD34$^+$: 5 $\times$ 10^5 cells transplanted

Analysis on 11th week after transplanting

FIG.12A

No of spleen cells = 2.52 × 10^6 × 5ml × 2



Gate	% Gated	% Total
G1	26.68	26.68
G3	16.30	16.30

Quad	% Gated	% Total
UL	43.26	11.54
UR	0.20	0.05
LL	56.36	15.04
LR	0.18	0.05

Quad	% Gated	% Total
UL	26.67	7.12
UR	16.80	4.48
LL	54.83	14.63
LR	1.71	0.46

FIG.12B

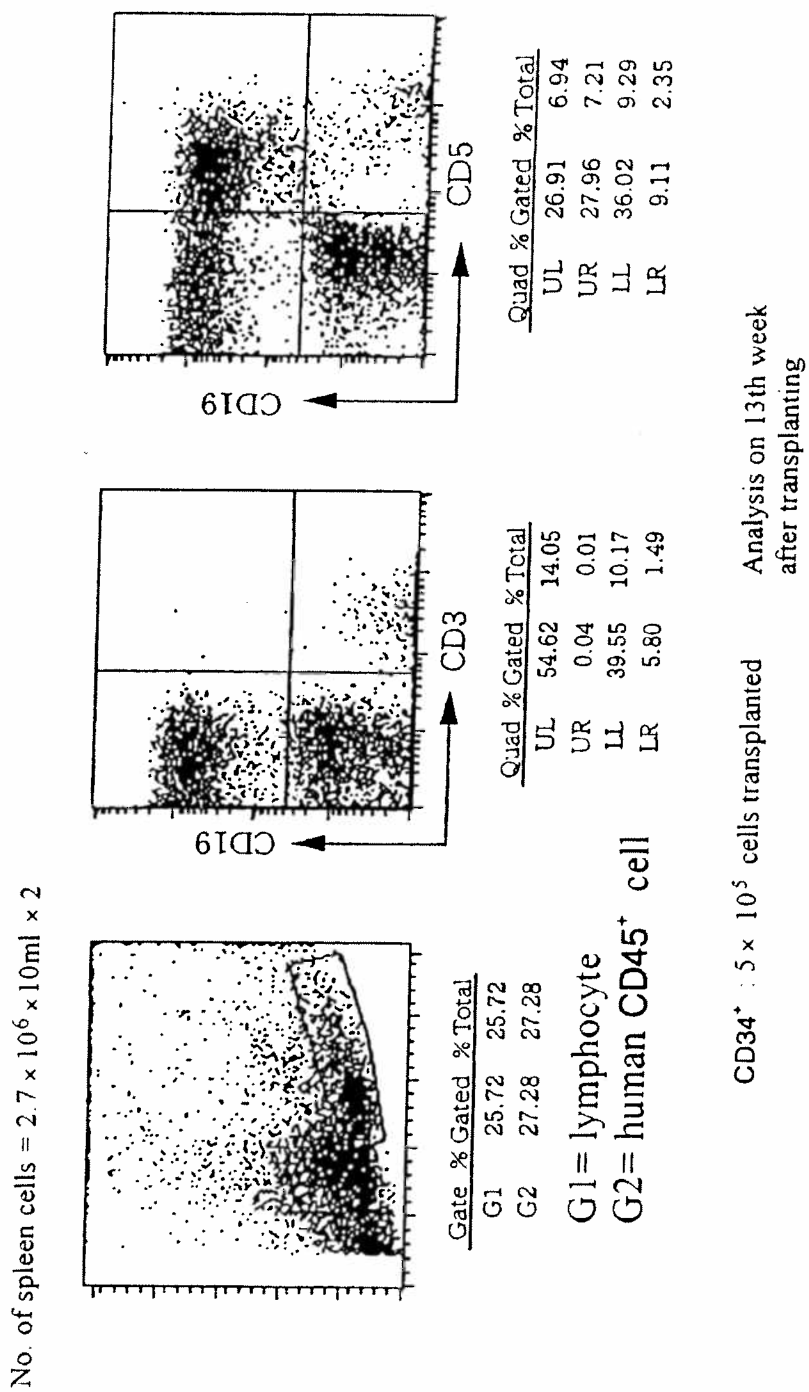
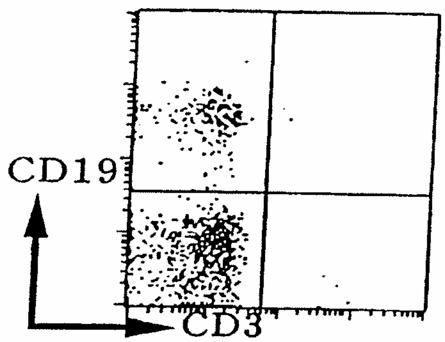

No. of spleen cells = 2.7 × 10^6 × 10ml × 2
Gate % Gated % Total
G1 25.72 25.72
G2 27.28 27.28
G1= lymphocyte
G2= human CD45+ cell
CD19
CD3
Quad % Gated % Total
UL 54.62 14.05
UR 0.04 0.01
LL 39.55 10.17
LR 5.80 1.49
CD19
CD5
Quad % Gated % Total
UL 26.91 6.94
UR 27.96 7.21
LL 36.02 9.29
LR 9.11 2.35
CD34+ : 5 × 10^5 cells transplanted
Analysis on 13th week after transplanting

FIG.13A

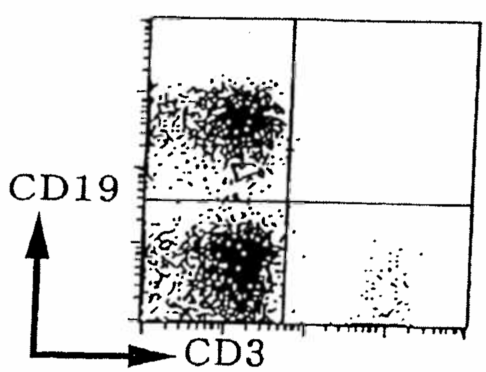
Flowcytometric Analysis of peripheral blood

G1= lymphocvte
G2= human CD45+ cell

11th week after transplanting CD34



Quad	% Gated	% Total
UL	21.46	0.22
UR	0.10	0.00
LL	77.97	0.81
LR	0.48	0.01



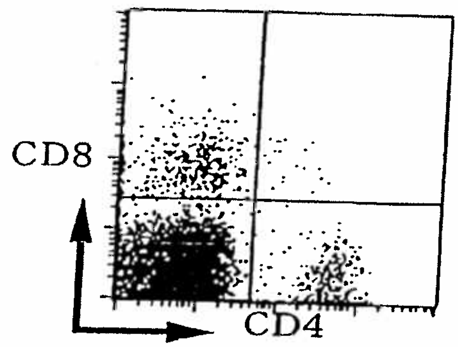
Quad	% Gated	% Total
UL	36.19	1.75
UR	0.06	0.00
LL	60.66	2.93
LR	3.09	0.15

FIG.13B

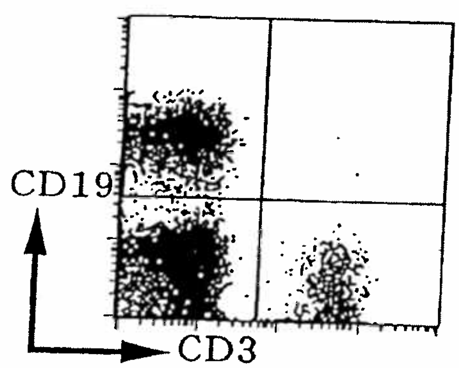
Flowcytometric Analysis of peripheral blood

G1= lymphocyte
G2= human CD45$^+$ cell

13th week after transplanting CD34



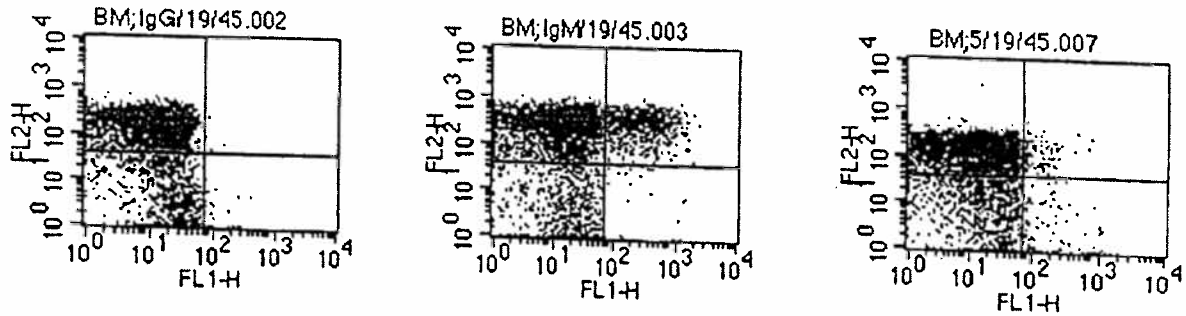
Quad	% Gated	% Total
UL	3.77	0.67
UR	0.18	0.03
LL	90.34	16.12
LR	5.72	1.02



Quad	% Gated	% Total
UL	31.41	3.40
UR	0.02	0.00
LL	55.11	5.97
LR	13.46	1.46

FIG.14A

Flowcytometric Analysis of bone marrow cells

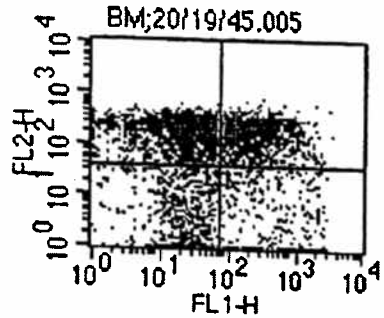


Quad	Events	% Gated	% Total
UL	7516	78.55	15.03
UR	4	0.04	0.01
LL	1998	20.88	4.00
LR	50	0.52	0.10

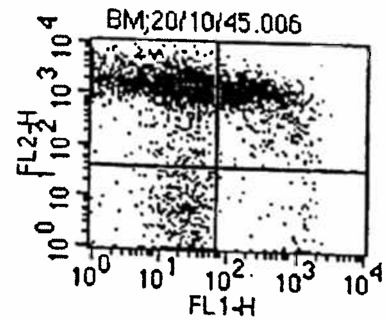
Quad	Events	% Gated	% Total
UL	6210	66.47	12.42
UR	1567	16.77	3.13
LL	1503	16.09	3.01
LR	62	0.66	0.12

Quad	Events	% Gated	% Total
UL	7174	75.35	14.35
UR	204	2.14	0.41
LL	1960	20.59	3.92
LR	183	1.92	0.37

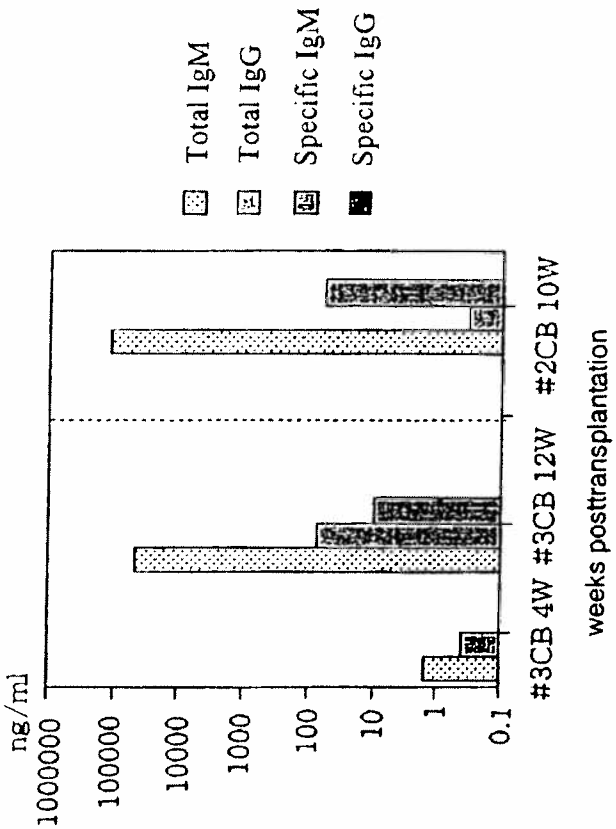
FIG.14B



Quad	Events	% Gated	% Total
UL	4347	47.17	8.69
UR	2683	29.12	5.37
LL	1545	16.77	3.09
LR	640	6.95	1.28

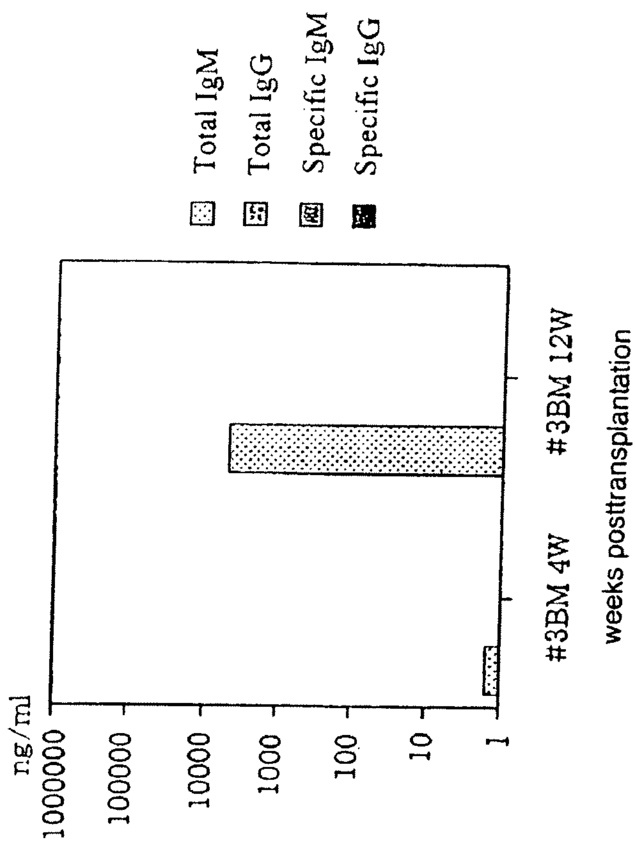


Quad	Events	% Gated	% Total
UL	5640	62.88	11.28
UR	2379	26.52	4.76
LL	813	9.06	1.63
LR	137	1.53	0.27


Total IgM
Total IgG
Specific IgM
Specific IgG
ng/ml
1000000
100000
10000
1000
100
10
1
0.1
#3CB 4W
#3CB 12W
#2CB 10W
weeks posttransplantation

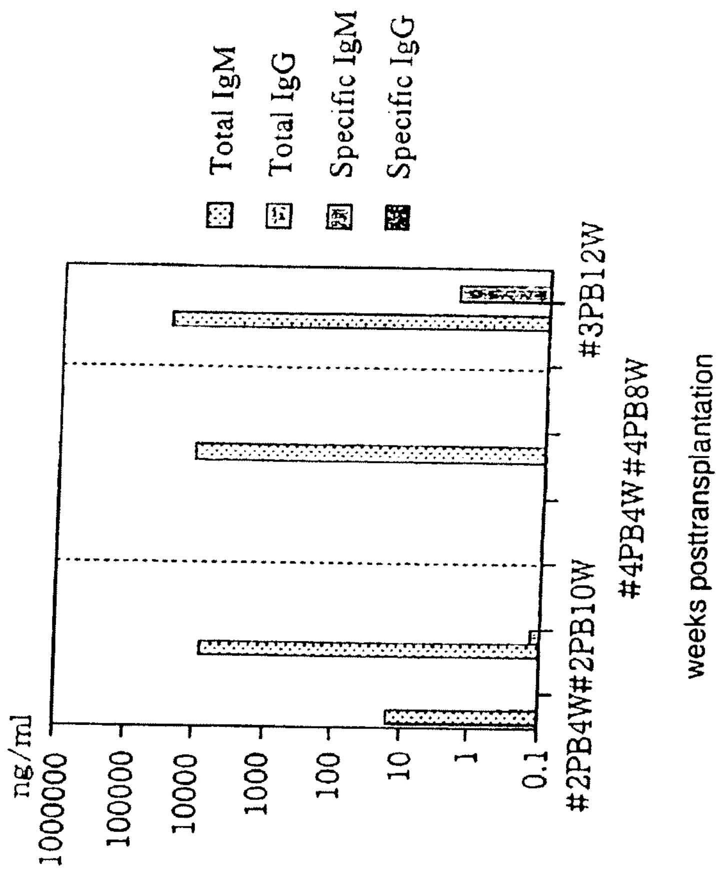
CB

FIG.15A


Total IgM
Total IgG
Specific IgM
Specific IgG
ng/ml
1000000
100000
10000
1000
100
10
1
#3BM 4W
#3BM 12W
weeks posttransplantation

BM

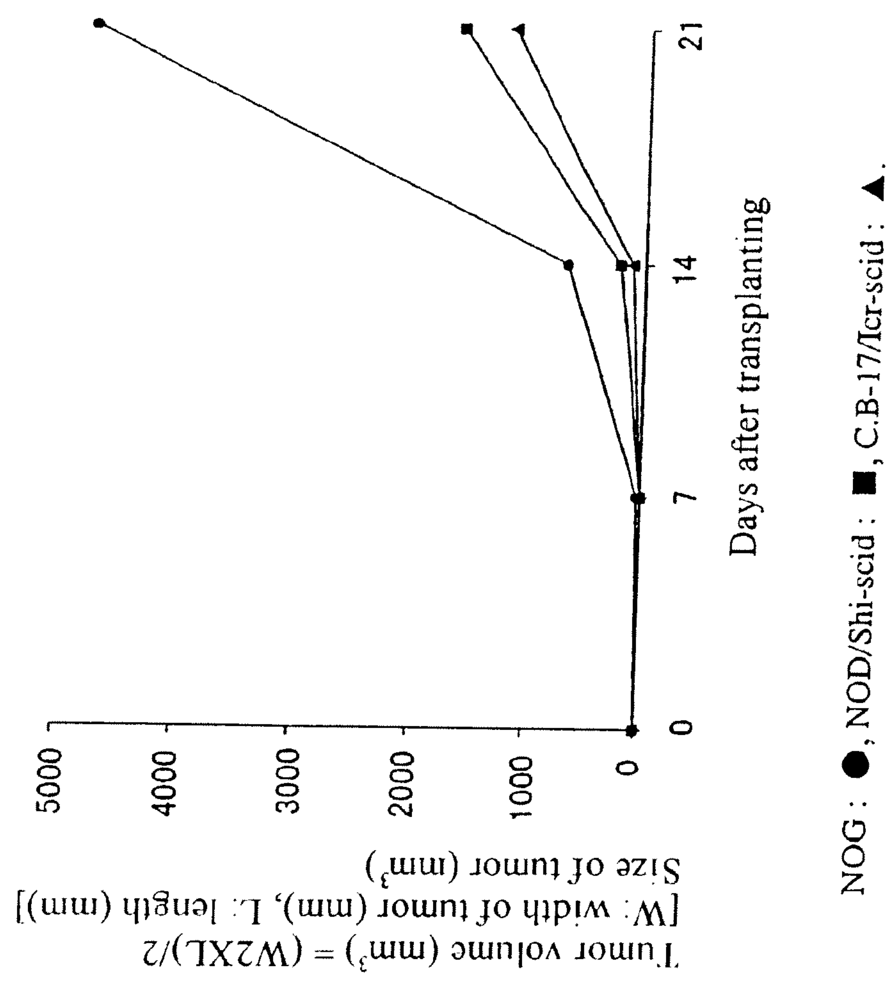
FIG.15B


Total IgM
Total IgG
Specific IgM
Specific IgG
ng/ml
1000000
100000
10000
1000
100
10
1
0.1
#2PB4W#2PB10W
#4PB4W#4PB8W
#3PB12W
weeks posttransplantation

PBSC

FIG.15C

FIG.16



Tumor volume (mm³) = (W2XL)/2
[W: width of tumor (mm), L: length (mm)]
Size of tumor (mm³)
5000
4000
3000
2000
1000
0
0
7
14
21
Days after transplanting
NOG : ●, NOD/Shi-scid : ■, C.B-17/Icr-scid : ▲.

METHOD OF PRODUCING A MOUSE SUITABLE FOR THE ENGRAFTMENT, DIFFERENTIATION AND PROLIFERATION OF HETEROLOGOUS CELLS, MOUSE PRODUCED BY THIS METHOD AND USE OF THE MOUSE

TECHNICAL FIELD

The present invention relates to a method of producing an excellent mouse for engraftment of heterologous cells, a mouse produced by this method and use of the mouse.

BACKGROUND ART

Laboratory animals to which heterologous cells including human cells are engrafted are very important for analysis of onset mechanisms of various diseases and drug developments for the treatments or preventions thereof, and development of animals as receptors therefor is one of major themes in laboratory animal sciences. In particular, in recent years, treatments etc. (known as regenerative medicine) in which tissues or cells differentiated from stem cells are transplanted have received world-wide attention, and therefore these animals are of increasing importance.

The inventors of the present invention have continued to develop and improve these laboratory animals. In particular, they made improvements or the like on a nude mouse or a SCID mouse, and they have already filed a patent application (Japanese Patent Application Laying-Open (kokai) No. 9-94040) concerning an immunodeficient mouse etc. produced for this purpose. Above all, an NOD/Shi-scid mouse and an NOD/LtSz-scid mouse which exhibit multifunctional immunodeficiency (functional deficiency of T cells and B cells, decline of macrophage function, reduction of complement activity, reduction of natural killer (NK) activity etc.) are the most noteworthy as laboratory animals suitable for engraftment of heterologous cells. Since it became clear that they could be used for various types of research including stem cell differentiation and proliferation, the range of applications in which they are used has increased to the present level.

However, human cells are engrafted to the NOD/Shi-scid mouse at a high ratio, but it is recognized that the engraftment capacity is substantially varied.

In order to enhance the engraftment capacity of the NOD/Shi-scid mouse, it has already been revealed that reduction of NK activity in the mouse by administering anti IL-2Rβ chain antibodies (TMβ1), anti-asialo-GM1 antibodies or the like is important. (Koyanagi, Y. et al., 1997. "Primary human immunodeficiency virus type 1 viremia and central nervous system invasion in a novel hu-PBL-immunodeficient mouse strain." J Virol 71:2417; Koyanagi, Y. et al., 1997. "High levels of viremia in hu-PBL-NOD-scid mice with HIV-1 infection." Leukemia 11 Suppl. 3:109; Yoshino H. et al., 2000. "Natural killer cell depletion by anti-asialo GM1 antiserum treatment enhances human hematopoietic stem cell engraftment in NOD/Shi-scid mice." Bone Marrow Transplant 26:1211-6. However, these antibodies are very expensive, and it is recognized that their efficacies vary between individuals. Further, when anti-asialo GM1 antibodies are used, the administration thereof should be conducted with the frequency of every eleventh day during the experiment period, and thus a degree of complexity is attached.

Therefore, Dr. Shultz, L. D. et al. of The Jackson Laboratory in the United States produced an NOD/LtSz-scid,

β2m null(β2m (null) NOD/SCID)mouse (Kollet O, Peled A, Byk T et al., beta2 microglobulin-deficient (β2m(null)) NOD/SCID mice are excellent recipients for studying human stem cell function. Blood 2000;95(10):3102-5) by crossing an NOD/LtSz-scid mouse having high engraftment capacity of human cells with a β2m KO mouse from which NK activity has been depleted.

With respect to the NOD/LtSz-scid, β2m null mouse, T cells, B cells and natural killer (NK) cells are depleted, and the function of macrophages and complements is reduced. However, other cells (e.g. dendritic cells) and factors (e.g. IFNγ) are also involved in the rejection of transplanted heterologous cells or tissues.

Accordingly, a mouse which, compared to the NOD/LtSz-scid, β2m null mouse, has no variation in heterologous cell engraftment capacity, requires no antibodies, and has excellent heterologous cell engraftment is desirable.

DISCLOSURE OF THE INVENTION

Thus, the object of the present invention is to solve the above problems and to provide a method of producing a mouse having excellent heterologous cell engraftment capacity and a mouse produced by the same method.

The present inventors have made intensive studies to solve the above problems. As a result, they have obtained the findings that a mouse which has no variation in engraftment capacity of heterologous cells and requires no antibodies (that is, is suitable for the engraftment of heterologous cells) can be obtained by backcrossing an NOD/Shi mouse with a C.B-17-scid mouse, and further backcrossing the thus obtained mouse with an interleukin 2-receptor γ-chain (IL-2Rγ) gene-knockout mouse. The present invention has been accomplished based on the above findings.

Namely, the present invention is as follows.

(1) A method of producing a mouse suitable for engraftment of heterologous cells, comprising backcrossing a mouse B with a mouse A, as described below:

A: a mouse obtained by backcrossing a C.B-17-scid mouse with an NOD/Shi mouse; and

B: an interleukin 2-receptor γ-chain (IL-2Rγ) gene knockout mouse.

(2) The method of producing a mouse as described in (1), wherein the mouse A is an NOD/Shi-scid mouse.

(3) The method of producing a mouse as described in (1) or (2), wherein the mouse B is an IL-2RγKO mouse.

(4) A mouse produced by the method of producing a mouse described in any of(1) to (3).

(5) A NOG (NOD/Shi-scid, IL-2Rγ KO) mouse having excellent engraftment capacity of heterologous cells, wherein both of functional T-cells and functional B-cells are deleted, macrophage function is reduced, NK cells or NK activity are eliminated, dendritic cell function is reduced.

(6) The NOG mouse described in (4) or (5), wherein transplanted human stem cells efficiently differentiate and proliferate without being eliminated.

(7) A stem cell assay method comprising transplanting human stem cells to the mouse described in any of (4) to (6) and analyzing the differentiated and proliferated cells.

(8) The stem cell assay method described in (7), comprising analyzing the differentiation and proliferation of T-cells and B-cells.

(9) A method of proliferating human stem cells comprising:

transplanting and proliferating the human stem cells to the mouse described in any of (4) to (6);

collecting the human stem cells from bone marrow of the mouse; and

repeatedly transplanting the collected cells to the mouse described in any of (4) to (6).

(10) The method of proliferating human stem cells described in (9), wherein the frequency of repeating is at least three times.

(11) Human stem cells obtained by the method of (9) or (10), wherein the obtained human stem cells have a purity of 99.7% or more.

(12) The method described in (9) or (10), wherein the human stem cells have foreign genes introduced thereinto.

(13) The mouse described in any of (4) to (6), wherein the mouse is capable of stably retaining human T-cells and B cells and producing a human antibody.

(14) A method of producing a human antibody comprising immunizing with an antigen the mouse described in any of (4) to (6) which retains human T-cells and B-cells.

(15) A method of producing an antibody-producing cell line which produces a human antibody, comprising:

immunizing with an antigen the mouse described in any of (4) to (6) which retains human T-cells and B-cells;

collecting from the mouse cells which produce the antibody against the antigen; and

establishing a cell line.

(16) A human tumor model mouse wherein the mouse is a mouse described in any of (4) to (6) and retains human tumor cells.

(17) The human tumor model mouse described in (16), wherein the human tumor cells are derived from HTLV-1 leukemia.

(18) The human tumor model mouse described in (16) or (17), wherein the mouse has the human tumor cells at an auricle thereof.

(19) A method of screening an anticancer agent using the mouse described in any of (16) to (18).

(20) A method of producing a human tumor model mouse comprising transplanting human tumor cells to the mouse described in any of (4) to (6).

(21) The method described in (16), wherein the human tumor cells are derived from HTLV-1 leukemia.

(22) The method described in (20) or (21), wherein the human tumor cells are transplanted at an auricle of the mouse.

(23) A virus-infected model mouse wherein the mouse is a mouse described in any of (4) to (6) and retains T cells infected with a T-tropic (T-cell affinity) virus as well as a macrophage-tropic virus.

(24) The virus-infected model mouse described in (23), wherein the virus is HIV.

(25) The virus-infected model mouse described in (23), wherein the virus is HTLV-1.

(26) A method of screening an antiviral agent wherein the method is carried out using the mouse described in (23) to (25).

(27) A method of producing an immunodeficient mouse which has engraftment capacity of enhanced heterologous cells compared with a NOG mouse, wherein the method is carried out using the mouse described in (3) to (6).

(28) The mouse described in (3) to (6), wherein the mouse is used for producing an immunodeficient mouse which has enhanced engraftment capacity of heterologous cells compared with a NOG mouse.

Hereinafter, general embodiments of the present invention will be described.

1. Production of a Mouse of the Present Invention

According to the present invention, a method of producing a mouse suitable for the engraftment of heterologous cells is characterized in backcrossing a mouse B with a mouse A, as described below.

A: a mouse obtained by backcrossing a C.B-17-scid mouse with an NOD/Shi mouse; and

B: an interleukin 2-receptor γ-chain gene knockout mouse.

Here, examples of the heterologous cells include cells or tissues derived from mammals such as humans, mice, rats etc., particularly human stem cells, lymphocytes or tumor cells etc. of humans, but not limited thereto.

With respect to the mouse A, backcrossing the C.B-17-scid mouse with the NOD/Shi mouse is done in accordance with methods well-known to a person skilled in the art, for example, backcrossing by Cross Intercross method (Inbred Strains in Biomedical Research, M. F. W. Festing, 1979, ISBN 0-333-23809-5, The Macmillan Press, London and Basingstoke). The C.B-17-scid mouse is crossed with the NOD/Shi mouse, and the obtained F1 mice are further crossed with each other. Then, the immunoglobulin amount in blood serum of the thus obtained F2 mice is measured for selecting a mouse, from which immunoglobulin cannot be detected. The selected mouse is again crossed with a NOD/Shi mouse. Repeating this process (Cross Intercross method) 9 times or more enables the accomplishment of the backcrossing.

A NOD/Shi mouse and a C.B-17-scid mouse are both commercially available from CLEA JAPAN, INC. Further, examples of mice obtained by crossing these mice with each other include a NOD/Shi-scid mouse (also called as a NOD-scid mouse) (Japanese Patent Application Laying-Open (kokai) No. 9-94040) which the present inventors have already established. This mouse is purchased from CLEA JAPAN, INC., and can be used directly as the mouse A. In addition, the present inventors possess, other than the ones mentioned above, NOD/Shi mice and NOD/Shi-scid mice, which can be split up and provided whenever the need arises.

Moreover, with respect to the mouse B, knockout of an interleukin 2-receptor γ-chain (IL-2Rγ) gene is carried out in accordance with methods well known to a person skilled in the art, for example, a homologous recombination method using mouse ES cells (Capecchi, M. R., Altering the genome by homologous recombination, Science, (1989) 244, 1288 1292). After substituting a specific mouse-derived gene by a homologous gene including a gene resistant to a drug, for example neomycin etc. at ES cell stage, the ES cells are inserted into a fertilized egg, thereby accomplishing the gene-knockout.

Specifically, for example, gene clones containing a mouse IL-2Rγ are isolated, from a genome library of 129/SV mouse, using a human IL-2RγcDNA as a probe. Using a fragment of 8.6 kb containing the full length of IL-2Rγ among the clones, a targeting vector is prepared. That is, PMC1-neo poly A which expresses a neomycin resistant gene, is inserted between exons 7 and 8 of IL-2R in the fragment, and also a diphtheria toxin-A gene is placed at 3' side 1 kb away from exon 8. Next, the vector is made linear, and introduced into 1×10^7 of E14 ES cells by electroporation. Thereafter, ES clones which bring about homologous recombination in the culture solution including G418, are selected (confirmed by PCR or Southern method), and after injecting the ES clones into blastocysts of C57BL/6 mice, they are transplanted into the uteruses of foster parent mice. Chimeric mice born from the foster parent mice are further

crossed with C57BL/6 mice, thereby obtaining IL-2RγKO hetero mice wherein knockout is transduced to germ cells.

Alternatively, pre-established interleukin-2 receptor γ chain gene (IL-2Rγ) knockout mouse strain may directly be obtained for use from suppliers, and examples of the mouse strains include interleukin-2 receptor γ chain (IL-2Rγ) knockout mice (Ohbo K, Suda T, Hashiyama M et al., Modulation of hematopoiesis in mice with a truncated mutant of the interleukin-2 receptor gamma chain. Blood 1996;87(3):956-67)) which was produced from IL-2RγKO mouse strains [Prof. Kazuo Sugamura, Department of Microbiology and Immunology, Tohoku University School of Medicine]. Incidentally, IL-2RγKO mice are presently stored in the embryo preservation bank of the applicants (Central Institute for Experimental Animals) at the request of Prof. Sugamura, a producer of the mouse strain, and whenever the need arises they can be provided as frozen embryos or as thaw-reconstruction mice.

Further, backcrossing the mouse B with the mouse A can be carried out, in similar fashion as described above, according to conventional methods well-known to a person skilled in the art. For example, in accordance with the above backcrossing, that is, a NOD/Shi-scid mouse is crossed with an IL-2RγKO mouse, and the obtained F1 mouse is backcrossed with an NOD/Shi-scid mouse, thereby accomplishing the backcross.

Furthermore, a mouse of the present invention is characterized in that the mouse is produced by the above method of the present invention. The mouse of the present invention is referred to as a NOG mouse (NOG mouse; NOD/Shi-scid, γc null mouse; NOD/Shi-scid, IL-2Rγ chain−/−mouse; NOD/Shi-scid, IL-2R(γc)^{null} mouse etc.)

The mouse of the present invention is a severe immunodeficient mouse in which has both of functional T-cells and functional B-cells are deleted, macrophage function are reduced, and NK cells or NK activity are eliminated. Therefore, when heterologous cells (e.g. human peripheral blood mononuclear leukocytes) are introduced into the mouse of the present invention, much higher ratios of engraftment and proliferation are observed even in comparison with conventional immunodeficient mice which are subjected to anti NK antibody treatment. (See Example 1 described below) Further, dendritic cells of the mouse of the present invention are also functionally incompetent, and the production of cytokine is remarkably reduced. Thus, the mouse of the present invention has the most excellent engraftment capacity of heterologous cells as compared with conventional immunodeficient mice, and it is considered effective for analyses of various introduced heterologous cells (including stem cells, differentiated cells and cancer cells) which are engrafted in this mouse. Additionally, it is possible to use the mouse in order to establish a pathologic model mouse and produce a human antibody for HIV, HTLV-1 or cancer.

Hereinafter, the applications of the mouse of the present invention will be described. Usually, the mouse to be used is preferably 8 to 12 weeks old, but not limited thereto.

2. Establishment of Human Stem Cell Assay System Using the Mouse of the Present Invention

Using the mouse of the present invention, it is possible to establish a human stem cell assay system for examining factors and mechanisms which are engaged in differentiation and proliferation of human stem cells. Also, it is possible to research various therapeutic products using the human stem cell assay system.

Introducing human stem cells into the mouse of the present invention enables the establishment of the human stem cell assay system. Here, stem cells include, in addition to hematopoietic stem cells, stem cells not derived from the hematopoietic system, such as neural stem cells etc. Human

stem cells are identified by the existence of a cell surface marker which relates to a specific epitope site identified by an antibody, and for example, they can be isolated as CD34 positive cells from e.g. human bone marrow, umbilical cord blood, peripheral blood etc.

Stem cells are suspended in a solution such as physiological saline, phosphate buffered physiological saline etc., which exerts no influence on cells and living organisms, and 1×10^4 to 1×10^6 of cells are intravenously administered into the mouse, thereby carrying out the transplantation.

Cells are collected, several weeks after transplanting, from each organ such as peripheral blood, the spleen, bone marrow, the thymus etc. of the mouse to which the cells have been transplanted. The surface antigens of these cells are examined using e.g. FACS (Fluorescence-activated cell sorter), and thereby the differentiation of the transplanted cells is examined. In this case, examples of cell surface antigen markers to be used as index include: CD34 which relates to stem cells; CD3, CD4, CD8 etc. which relate to T-cells; CD10, CD19, CD20 etc. which relate to B-cells; CD5 etc. which relate to B_1a cells; CD33 etc. which relate to myeloid cells; CD11c etc. which relate to dendritic cells; CD45 etc. which relate to the whole leukocytes; CD11a, CD11b etc. which relate to macrophages; CD56 etc. which relate to NK cells; CD38 etc. which relate to plasma cells; CD41 etc. which relate to platelets; and glycophorin A etc. which relate to erythrocytes. According to need, various related markers can be selected.

The production of cytokines such as interferon, interleukin, TNFα etc. in the collected cells, is measured by ELISA etc., and thereby the differentiation of the stem cells is examined.

Further, it is possible to conduct successive transplantations of human stem cells using the mouse of the present invention. That is, true self-replicable human stem cells can be obtained. Specifically, human stem cells are transplanted in the mouse of the present invention, after several weeks undifferentiated human stem cells are collected from bone marrow of the mouse, and further the collected stem cells are transplanted in the mouse of the present invention. By repeating the transplantation and collection, human stem cells which are free from other cells and have high purity can be obtained in large quantities. By the successive transplantations, human stem cells with at least 99% or more purity, preferably 99.7% or more purity can be obtained. Conventional mice allow up to secondary transplantation, though the mouse of the present invention enables more than two successive transplantations.

For the treatment of leukemia or the like, human stem cells obtained using the mouse of the present invention can be transplanted to humans. Also, the mouse is usable for gene therapy targeting human stem cells by introducing a foreign gene into human stem cells and transplanting them to the mouse of the present invention for proliferation. With the aid of virus vectors such as lentivirus vectors, retrovirus vectors, adenovirus vector, and adeno-associated virus vector, a gene can be introduced into stern cells. Examples of the genes to be used here include an ADA gene for adenosine deaminase deficiency (ADA) patients. After these genes are introduced into human stem cells, the cells are transplanted to the mouse of the present invention for proliferation and purification and then administered to patients, thereby enabling gene therapy.

3. Production of Human Antibodies Using the Mouse of the Present Invention

Using the mouse of the present invention, established human cell lines which produce human antibodies, and human antibodies can be obtained. The above-described stem cells are transplanted to the mouse of the present

invention, and cells responsible for immunity such as T-cells or B-cells are differentiated and proliferated. Alternatively, cells responsible for immunity such as human T-cells or B-cells are transplanted to the mouse and engrafted in the mouse body, and thereby obtained is a mouse having the cells responsible for human immunity and capable of producing human antibodies. When human stem cells are transplanted, the differentiation and proliferation of T-cells and B-cells are realized in 6 to 8 weeks, enabling the production of human antibodies.

By administering antigens to the mouse having human T-cells and B-cells engrafted thereto and held, it is possible to obtain human antibodies against the antigens and cells capable of producing the antibodies. The administration of the antigens to the mouse of the present invention may be carried out by the same method as is conventionally used for immunizing a mouse.

Human antibody producing cells can be collected from each organ of the mouse, especially the spleen, lymph nodes etc. When the ratio of the human antibody producing cells is high, the collected cells can directly be used for establishing a cell line. However, when the ratio is low, if necessary, purification may be carried out by e.g. the affinity column method using anti human B-cell antibodies. Further, it is desirable to eliminate mixed-in mouse cells by e.g. cytolysis method using anti mouse antibodies and complements.

The thus obtained human antibody producing cells are made into a established line by a transformation method using Epstein-Barr virus (EBV), a cell fusion method wherein the cells are fused with suitable proliferation viable cells, or the like. Then, obtainable is a human antibody-producing established cell line capable of multiple passages while producing antibodies.

4. Production of a Pathologic Model Mouse with Tumor

In the mouse of the present invention, human tumors can be engrafted and proliferated, and an animal model of a human tumor can be obtained by transplanting tumor cells to the mouse of the present invention. For example, the administration of the human tumor cells causes the proliferation thereof inside the mouse body, and thus a mouse having a human tumor can be obtained. Examples of the cells to be used in this case include subcultured lines of human tumor in a conventional nude mouse, and cell lines derived from HTLV-1 leukemia such as ED-40515(−), MT-1 and TL-Om1. In addition, human tumor tissues are chopped into pieces having a size of several mm, and these cancer tissue pieces may directly be transplanted and engrafted to the mouse of the present invention. In this case, the site of the mouse to which tumor cells or tissues are transplanted is not limited, but in the case of cells, they may be transplanted intraperitoneally, intravenously or subcutaneously to the mouse and in the case of the tissues, they may be transplanted subcutaneously to the mouse. Any subcutaneous site of the mouse may be acceptable such as subcutaneous gluteal region, but it is desirable to transplant them subcutaneously at an auricle or a dorsal region because the tumor can be checked without incision. Further, in order to obtain results which reflect clinical effects of an anti cancer agent, it is desirable to transplant them at the identical site as that for clinical test (in the case of colon cancer cells, the cells are to be transplanted to the colon). When the cells are transplanted, a tumor is formed within several weeks to several months. Specifically, when HTLV-1 cells are transplanted subcutaneously at a posterior auricle, a tumor is formed in 2 weeks, thereby enabling expeditious production of a practical tumor model mouse.

Moreover, when a tumor is transplanted to the mouse of the present invention, metastases of tumor cells such as leukemic changes are observed and the mouse is usable as a model animal for tumor metastasis.

Using the thus obtained human tumor model mouse, screening of an anti cancer agent, antimetastatic drug etc. can be performed. As a method therefor, a candidate agent is administered to a mouse having a tumor formed, by a suitable method e.g. oral, transdermal administration or the like. Then, observation on the size of the tumor, the size and number of metastatic focuses, the viability of the mouse etc., allows judgment on the effect of the drug.

5. Production of a Viral Infectious Disease Model Mouse

Use of the mouse of the present invention enables the obtainment of a viral infectious disease model mouse. Namely, by transplanting to the mouse of the present invention cells which may be infected with a human virus, and infecting the cells with the virus, or transplanting cells infected with a virus, it is possible to obtain a viral infectious disease model animal, which has virus-infected cells engrafted and held.

In a conventional mouse, only an M-tropic virus which infects macrophages can proliferate, but the proliferation of T-tropic viruses such as HIV, HTLV-1, which infects T-cells becomes possible using the mouse of the present invention.

For example, 1×10^7 to 1×10^8 of human peripheral blood mononuclear leukocytes are intraperitoneally administered to the mouse of the present invention, and after several days some hundreds to thousands of $TCID_{50}$ of HIV were inoculated, thereby obtaining a HIV-infected model mouse having human cells infected with HIV. The HIV infection can be detected through the expression of HIV antigens such as p24 positive cells as an index.

Instead of HIV, the inoculation of HTLV-1 enables the obtainment of an HTLV-1 infected model mouse.

Use of the animal model for disease obtained according to the present invention, allows in vivo research on proliferation mechanisms of HIV, HTLV-1 etc., further development of therapies for virus infections, screening of therapeutic products for virus infection, or the like.

6. Production of a Mouse Having Enhanced Engraftment Capacity of Heterologous Cells Using a NOG Mouse

Use of the NOG mouse of the present invention enables the production of a mouse having more enhanced heterologous cell engraftment. For example, such a mouse can be obtained by backcrossing a mouse wherein a gene relating to the mouse immune system is knocked out with the NOG mouse of the present invention. Examples of the genes relating to the immune system include cytokine receptor gene, cytokine gene etc.

Further, by introduction of human cytokine gene, which relates to the differentiation and proliferation of human cells, or the like (e.g. hGM-CSF or hSCF etc.), it is possible to produce a mouse having more enhanced heterologous cell engraftment. For instance, in accordance with a method of Pro. Natl. Acad. Sci. USA 77:7380 7384, 1980, or the like, the above gene is inserted into a pronuclear fertilized egg of the mouse, and an individual having this introduced gene incorporated thereinto is selected, thereby producing a mouse which expresses a human cytokine gene etc. Then, this mouse and the NOG mouse of the present invention were crossed with each other, thereby producing a mouse having enhanced heterologous cell engraftment.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows an outline of backcrossing for producing a NOG mouse.

FIGS. 2A and 2B show time-course changes of human CD45 positive cells and human CD41 positive cells, after the introduction thereof, in peripheral blood of the NOG mouse to which CD34 positive cells are transplanted.

FIG. 3 shows the ratio of CD45 positive cells in the bone marrow and spleen of the NOG mouse to which CD34 positive cells are transplanted.

FIG. 4 shows the ratios of CD45 positive cells in peripheral blood of mice in a comparative test between the NOG mouse and a β2 microglobulin deficient NOD-SCID mouse (NOD/LtSz-scid, β 2 m null mouse).

FIGS. 5A and 5B show FACS patterns of NK cells and dendritic cells in spleen cells obtained from each mouse strain.

FIGS. 6A, B and C show the results of ELISA for detecting the production amount of cytokine under the stimulation of Listeria monocytogenes antigens in spleen cells obtained from each mouse strain.

FIG. 7 shows the removal of NK activity in the NOG mouse and the NOD/LtSz-scid, β 2m null mouse.

FIG. 8 shows the results of FACS wherein bone marrow cells from primary, secondary and tertiary mice to which human CD34 positive cells have been transplanted, are stained with human CD45.

FIGS. 9A and 9B show engraftment and differentiation of human cells in the thymus of the OG mouse to which human CD34 positive cells have been introduced.

FIGS. 10A and 10B show engraftment and differentiation of human cells in the thymus of the NOG mouse to which human CD34 positive cells have been introduced.

FIGS. 11A and 11B show engraftment and differentiation of human cells in the spleen of the NOG mouse to which human CD34 positive cells have been introduced.

FIGS. 12A and 12B shows show engraftment and differentiation of human cells in the spleen of the NOG mouse to which human CD34 positive cells have been introduced.

FIGS. 13A and 13B show engraftment and differentiation of human cells in peripheral blood of the NOG mouse to which human CD34 positive cells have been introduced.

FIGS. 14A and 14B show engraftment and differentiation of human cells in bone marrow of the NOG mouse to which human CD34 positive cells have been introduced.

FIGS. 15A, B and C show the ability of NOG mouse transplanted with umbilical cord blood (CB), bone marrow (BM) and peripheral blood stem cells (PBSC), respectively, to produce human antibodies.

FIG. 16 shows the tumor formations after transplanting LM-2-JCK to each mouse strain.

BEST MODE FOR CARRYING OUT THE INVENTION

Next, the present invention will be described in detail by referring to Examples.

EXAMPLE 1

Production of an Immunodeficient Mouse (NOG Mouse) with the Deletion of NK Activity and Declined Dendritic Cell Function, Examination of the Heterologous Cell Engraftment in the Mouse, and Establishment of an Assay System of Human Stem Cells Using the Mouse

(1) Production of an Immunodeficient Mouse (NOG Mouse) With the Elimination of NK Activity and Reduced Dendritic Cell Function

In order to obtain multifunctional immunodeficient mice with depleted NK activity, interleukin-2 receptor γ chain knockout mice (IL-2RγKO mice) (8 week-old) which were transferred from Prof. Kazuo Sugamura (Department of Microbiology and Immunology, Tohoku University, School of Medicine) were backcrossed with NOD-Shi-scid mice (8 week-old) which were kept in the Central Institute for Experimental Animals (also available from CLEA JAPAN, INC.), and thereby F1 mice having an IL-2Rγ mutant gene introduced thereinto were produced. The introduction of the mutant IL-2Rγ chain gene in the F1 mice was confirmed by PCR amplification and detection of the gene. Specifically, first, DNAs were extracted by a DNA automatic extractor (MagExtractor manufactured by TOYOBO) from 100 μl of blood taken from ocular fundus of the F1 mice. PCR buffer solution containing 23.5 μl of 1.5mM $MgCl_2$, 0.4 mM dNTP and two sets of 25 pmol primers (the following primers PI and PIII were used for the determination of wild type, and a set of the following primers PI and PII were used for the determination of mutant type) was added to 1.5 μl of this DNA, and PCR was conducted under the following amplification conditions for the determination of whether IL-2Rγ chain genes were wild type or mutant type.

(Primers)

PI	5'-CTGCTCAGAATGATGCCTCCAATTCC-3'	SEQ ID NO:1
PII	5'-CCTGCGTGCAATCCATCTTGTTCAAT-3'	SEQ ID NO:2
PIII	5'-GATCCAGATTGCCAAGGTGAGTAG-3'	SEQ ID NO:3

(PCR Amplification Conditions)

The conditions were heating at 94° C. for 5 minutes; 30 to 35 cycles of 1 minute at 94° C., 1 minute at 55° C., and 1 minute at 72° C.; and thereafter heating at 72° C. for 10 minutes.

The PCR products obtained by the above PCR were subjected to electrophoresis in 2% agarose gel, and measured according to the size of the coloring band detected after ethidium bromide stain. The sizes of the bands, about 660 bp for wild type and about 350 bp for mutant type, were observed.

(Backcrossing)

Next, the F1 mice having the mutant IL-2Rγ gene introduced thereinto were crossed with NOD/Shi-scid mice, thereby obtaining F2 mice. Further, by detecting the introduction of the mutant IL-2R γ chain gene into the F2 mice in the same manner as above, and detecting immunoglobulins in serum by an immunodiffusion method, mouse individuals which had the mutant IL-2R γ chain gene and had a homozygous scid gene were selected. Thereafter, the mouse individuals were crossed with NOD/Shi-scid mice, and among the born mice, mice having mutant IL-2R γ chain gene were further crossed with NOD/Shi-scid mice.

The above backcross was repeated at least 9 times, thereby producing NOG (NOG) mice (FIG. 1 shows the outline). Here, since the IL-2R γ chain gene exists on an X chain chromosome, it is effective to use male IL-2RγKO mice.

(2) Examination on Engraftment Capacity of Heterologous Cells in NOG Mice

Next, using the NOG mice obtained by the above crossing and conventional immunodeficient mice, NOD/Shi-scid mice, examinations were made on the level of impact that anti-NK antibody treatment has on engraftment of heterologous cells in these mice.

(Anti-IL-2 Receptor β Chain Monoclonal Antibody)

Anti-IL-2 receptor β chain monoclonal antibodies (clone TM β1) were produced from hybridomas produced and provided by Prof. Masayuki Miyasaka, School of Medicine, Osaka University (Tanaka T, Tsudo M, Karasuyama H et al., A novel monoclonal antibody against murine IL-2Receptor beta-chain. Characterization on of receptor expression in normal lymphoid cells and EL-4 cells. J Immunol 1991;147 (7):2222 8). In particular, the hybridomas were intraperito-